

INVESTIGATING GROUNDWATER ARSENIC CONTAMINATION USING  
AQUIFER PUSH-PULL TESTS

by

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A THESIS

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“Investigating Groundwater Arsenic Contamination using Aquifer Push-Pull Tests,” a thesis prepared by Ashley R. Daigle in partial fulfillment of the requirements for the Master of Science degree in the Department of Geological Sciences. This thesis has been approved and accepted by:

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An Abstract of the Thesis of  
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Title: INVESTIGATING GROUNDWATER ARSENIC CONTAMINATION USING  
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The bedrock aquifer of the Southern Willamette Basin, Oregon, USA, is contaminated with arsenic at concentrations as high as several ppm. Single-well push-pull tests were conducted to investigate how microbial metabolisms control arsenic occurrence and levels in the aquifer. Test solutions containing ethanol were injected into the aquifer; dissolved gases, groundwater, and sediments were then sampled to monitor the speciation of carbon, iron, sulfur, and arsenic. Ethanol amendment stimulated a series of microbial metabolisms, including arsenate reduction, iron reduction, and sulfate reduction. Arsenate reduction converts arsenate to arsenite; iron reduction produces ferrous iron; sulfate reduction releases sulfide. Arsenite and ferrous iron then combine with sulfide and form arsenic sulfide and iron sulfide minerals. Results of the experiments demonstrate that the interactions among microbial metabolisms and mineral

precipitation influenced arsenic contamination in the aquifer. These results shed new light on potential bioremediation strategies in the area.

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## CHAPTER I

### INTRODUCTION

Arsenic (As), a common natural and anthropogenic contaminant, is ubiquitous in natural environments. Its occurrence in water supplies presents a health threat, causing serious dermatological conditions and damaging the cardiovascular, respiratory, gastrointestinal, and nervous systems (Hopenhayn, 2006). Due to the significant health impact of arsenic, the U.S. Environmental Protection Agency lowered the maximum contaminant level for arsenic in drinking water from 50 ppb to 10 ppb in 1996. Several potential mechanisms have been proposed to explain groundwater arsenic contamination, including microbial reduction of As-bearing iron oxyhydroxides (Harvey *et al.*, 2002), desorption by competing ions (Manning and Goldberg, 1996; Livesey and Huang, 1981; Swartz *et al.*, 2004), and high pH (Smedley and Kinniburgh, 2001). However, despite the severe health impact of arsenic, the processes and environmental conditions responsible for mobilizing arsenic in groundwater remain relatively unknown (Polizzotto *et al.*, 2005).

Arsenic is redox sensitive and occurs in groundwater mainly as arsenate,  $\text{As}^{5+}$ , and arsenite,  $\text{As}^{3+}$  (Massachusetts *et al.*, 1991). Both abiotic reactions and microbial metabolisms can reduce arsenate to arsenite; however at  $\text{pH} > 7$ , no significant abiotic reduction was observed by Rochette *et al.* (2000). Microorganisms can also detoxify arsenic by converting arsenate and arsenite to organic arsenic species, such as

methylarsonate (MMA) and dimethylarsinite (DMA), which degas out of solution (Cullen and Reimer, 1989).

Both arsenate and arsenite adsorb onto the mineral surfaces of aquifer sediments, specifically iron oxides and clays (Welch and Lico, 1998). The sorption of arsenic is highly pH dependent with arsenate sorbing significantly at a pH less than 6, while arsenite preferentially sorbs at a pH greater than 8 (Smedley and Kinniburgh, 2001). The sorption tendencies of organic arsenic species are relatively unclear. In general, as arsenic becomes more methylated, its tendency to adsorb decreases (Lafferty and Loeppert, 2005).

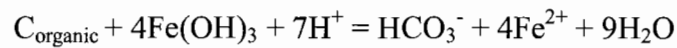
Arsenic redox speciation also has toxicological implications. Morrison *et al.* (1989) reported that arsenite is 50 times more toxic than arsenate. Hindmarsh (2007) determined that both arsenate and arsenite catalyze the formation of tumors within the bladder, lungs, liver, and kidneys. These devastating effects occur at concentrations of >200 ppb, but concentrations of <100 ppb have shown no known direct effects (Hindmarsh, 2007). Arsenite is also more difficult to remove from drinking-water than arsenate (Schneiter and Middlebrooks, 1983). Redox transformation between arsenate and arsenite in the environment thus is one of the most important processes controlling arsenic adsorption, mobilization, and toxicity in aquifer systems (Rhine *et al.*, 2001).

Microbial metabolisms catalyze the transformation of carbon, iron, sulfur, and arsenic in pristine aquifer systems. Table 1 shows the different microbial metabolisms, i.e., iron reducers, sulfate reducers, arsenate reducers, and methanogens, observed in the aquifer and their impacts on groundwater chemistry.

TABLE 1. Microbial metabolisms and their respective impact on groundwater chemistry.

Metabolism	Impact
Fe(III) reduction	Fe(II) accumulation
Sulfate reduction	Sulfide accumulation
Arsenate Reduction	Arsenite accumulation
Methanogenesis	Methane accumulation

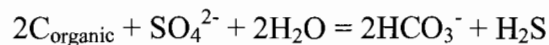
From a geochemical point of view, Fe(III) reduction is one of the most important microbially-mediated processes of a pristine aquifer system. Iron reducers convert ferric iron, Fe(III), to ferrous iron, Fe(II). Stoichiometrically, the reaction is



(Drever, 1997). Sulfate reduction is another important microbially-mediated process.

Sulfate reducing microbes use  $\text{SO}_4^{2-}$  as a terminal electron acceptor in the oxidation of organic matter to  $\text{CO}_2$ , forming sulfide ( $\text{H}_2\text{S}$ ) as a byproduct (Madigan *et al*, 2000).

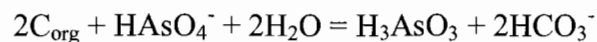
Stoichiometrically, the reaction is



(Drever, 1997). Though not globally common, microbially-mediated arsenate

reduction also occurs in arsenic-contaminated aquifers, converting arsenate to arsenite

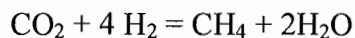
(Hoeft *et al.*, 2004):



Methanogenesis is the final step in the degradation of organic matter. The microbes that perform methanogenesis are known as methanogens. Common methanogenesis pathways in aquifers include hydrogenotrophic and acetoclastic (Waldron *et al*, 2007).



Stoichiometrically, where  $H_2$  is utilized as the electron donor, hydrogenotrophic methanogenesis proceeds according to



For acetoclastic methanogenesis, the reaction proceeds according to



Microbial metabolisms do not occur individually, but are intertwined with each other in aquifers. Where microbial iron reduction overlaps with sulfate reduction, Fe(II) may precipitate with sulfide as sulfide minerals, such as amorphous iron sulfide (Drever, 1997). Similarly, where microbial arsenate reduction occurs simultaneously with sulfate reduction, arsenite may precipitate with sulfides, producing amorphous arsenic sulfide. Microbial metabolism and associated geochemical reactions (e.g., sorption and mineral precipitation) thus control significantly groundwater arsenic redox speciation, which in turn controls arsenic concentration and mobilization in arsenic-rich aquifers. Oremland and Stolz (2003) believe that “there is an immediate research need for a full understanding of the role of subsurface microbes in mobilizing arsenic in aquifers”. The goal of this thesis research thus is to evaluate the significance of microbial metabolisms in controlling the occurrence and levels of arsenic in groundwater.

One challenge in the understanding microbial metabolisms in aquifers is the sluggish progress of the metabolism due to the limited availability of electron donors. For example, the rates of microbial metabolisms are commonly at the order of  $10^{-6}$  -

$10^{-9}$  molal/day (Chapelle, 2001). Thus, it is impractical to characterize the occurrence and impact of microbial metabolism under natural conditions.

To investigate microbial metabolism and its impact on arsenic under *in situ* conditions, push-pull tests were conducted using ethanol as an electron donor. The push-pull test consists of two phases, i.e., a phase of test solution injection, the push, followed by groundwater monitoring, the pull (Istok *et al.*, 2004). After the test solution is injected into the aquifer, groundwater was sampled periodically for chemical analysis. The amendment of ethanol stimulates microbial metabolism, which allows the direct observation and quantification of microbial metabolism and resulting geochemical reactions in the aquifer. Although the rates of microbial metabolism stimulated by ethanol amendment are orders of magnitude larger than the rates under natural conditions, the potential mechanisms controlling arsenic contamination revealed by the push-pull tests are equally applicable to the aquifers under natural conditions; because the microbial metabolisms studied occur naturally, simply at lower rates.

Results of this study quantitatively describe the significance of different microbial groups in regulating arsenic concentrations in aquifers. This study will also provide government agencies with critical information concerning groundwater microbiology allowing for effective strategies and regulations for groundwater resource management, monitoring, and protection.

## CHAPTER II

### FIELD EXPERIMENTS

Two push-pull tests were conducted to test the hypothesis that **microbial metabolisms significantly control arsenic speciation in groundwater, which in turn controls the occurrence and mobility of arsenic**. The push-pull tests were conducted in the bedrock aquifer of the Southern Willamette Basin, Oregon, USA (Fig. 1). Groundwater flows from the recharge area in the hills, where arsenic concentrations are low, down into the valley, where over the flow path arsenic concentrations increase (see Fig. 1). The bedrock aquifer consists of a terrestrial volcaniclastic mudflow deposit and tuff (Fisher Formation) and marine arkosic sandstone (Eugene Formation) (Murray, R.B., 2005). Groundwater in the aquifer is neutral to slightly alkaline (pH from 7 to 9) and is anoxic (Hinkle and Polette, 1999). Arsenic occurs in the groundwater mainly as arsenite at concentrations as high as 2300 ppb (see Table 2), which grossly exceeds the 10ppm U.S. EPA limit for drinking water.

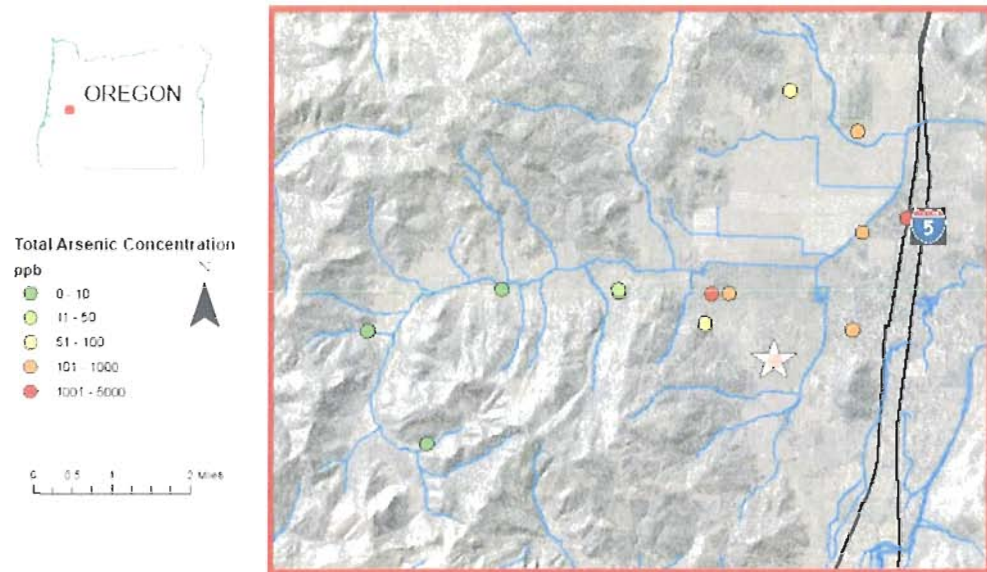


FIGURE 1. Location of the study area in the Southern Willamette Basin, Oregon, USA. The star indicates the location of the test well.

TABLE 2. Chemical composition of the test solution and groundwater before the onset of the test (pretest) during the control push-pull test.

		Test Solution	Pretest
Volume	(gal)	40	n.a.
T	(°C)	19.2	15.0
pH		7.0	7.6
Conductivity	( $\mu$ S)	1233	2090
Alkalinity	(mM)	11.83	12.40
Chloride	(ppm)	100.73	617.37
Ferrous iron	(ppm)	0.3	1.1
Sulfate	(ppm)	132.8	244.3
Arsenite	(ppb)	28	2300
Arsenate	(ppb)	1900	130

n.a., not applicable.

## 2.1. Experiment Setup

A pair of inflatable stainless steel packers was installed in the test well to isolate a 10 ft interval where the test solution entered the aquifer. The packers were connected to the ground surface using PVC pipes (2 inches in diameter and 10 feet in length). The test solution was pumped into the test zone using a peristaltic pump. Groundwater samples are collected from the test zone using an S.S. Mega-Monsoon 12 Volt pump (Proactive Environmental Products).

## 2.2. Transmissivity

To locate the interval of the well borehole where the test solution can enter the aquifer, a series of slug tests were conducted to analyze the transmissivity of the aquifers around the well borehole. The ideal depth interval for the push-pull test is the interval isolated by the packer that has a low connectivity with the outside of the packer, but a large transmissivity. The transmissivity is a measure of the quantity of water that the aquifer can transmit horizontally through a unit width by the full saturated thickness under a hydraulic gradient of 1 (Fetter, 1994). By using water-level meters both inside and outside the PVC pipe within the well borehole and pumping at a high rate (4.2 L/min), a rate of drawdown (the decreases in hydraulic head by pumping) both inside and above the packer were recorded along with corresponding recovery rates of the hydraulic head once pumping ceased (see Figure 2). Boreholes at depths of 85, 90, 95, 100, 105, 110, 115, 120, and 125 feet (measured from the ground surface to the middle of the perforated zone of the packer) were tested. Using the Jacob straight-line method (Fetter, 1994), transmissivity (mL/m) is calculated:

$$T = \frac{2.3Q}{4\pi\Delta S} \quad (\text{eq. 1})$$

where  $Q$  is the pumping rate (mL/min) and  $\Delta S$  is the slope of drawdown vs. log time (m/min). Connectivity,  $C$ , between the section of well borehole isolated by the packer and that above the packer is calculated:

$$C = \frac{T_{\text{inside}}}{T_{\text{outside}}} \quad (\text{eq. 2})$$

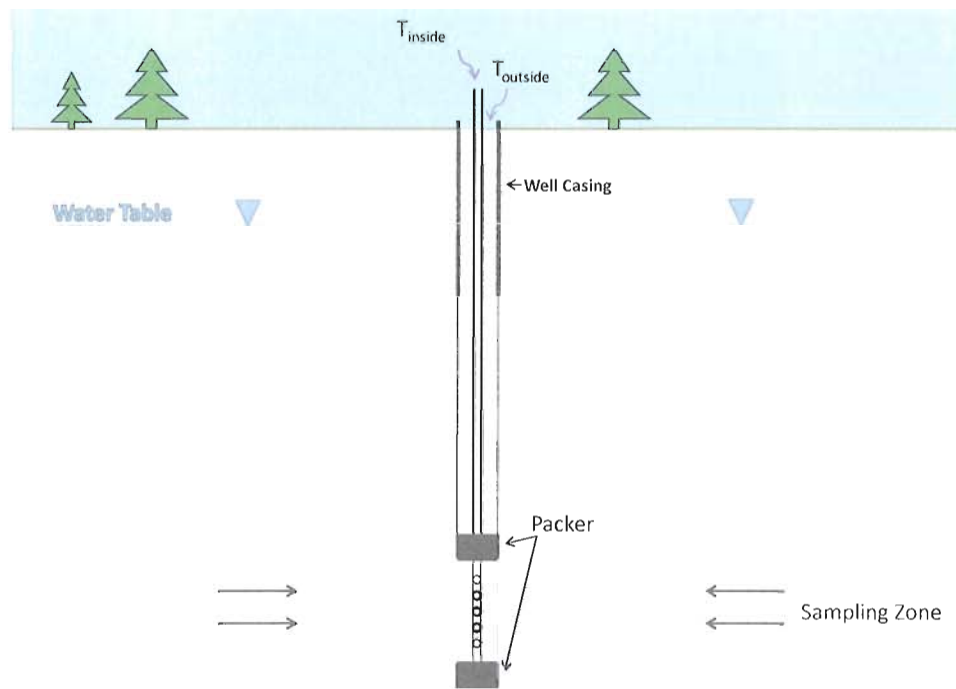


FIGURE 2. Schematic depicting the method for testing transmissivity.

### 2.3. Control Experiment

The test solution was prepared using 40 gallons of water from a nearby well in a 100 gallon carboy. The pH was adjusted to 7.5 using potassium hydroxide (KOH). Sodium bicarbonate ( $\text{NaHCO}_3$ ) was added to a final concentration of 10 mM, and arsenate was added, in the form  $\text{Na}_2\text{HASO}_4$ , to a final concentration of 1 ppm to verify the occurrence of microbial arsenate reduction. Table 2 shows the concentrations of

chemical species in the test solution. This solution was purged with 95% N<sub>2</sub> and 5% CO<sub>2</sub> until the dissolved oxygen content was below the detection limit of the DO meter (YSI, Inc, Model: 55-12FT), so no oxygen was introduced into the anoxic aquifer. The test solution was then pumped between the packers using a peristaltic pump at a rate of ~2 gallons per minute. The water level in the packer was monitored; the increases in the water level were less than 2 ft during the injection.

#### 2.4. Ethanol-amended Experiment

The test solution was similar to that for the control experiment, except that bromide was added as the conservative tracer to a concentration of 100 ppm Br<sup>-</sup> and organic matter in the form of ethanol was added to a concentration of 40 mM (see Table 3). The experiment procedure was the same as discussed above.

TABLE 3. Chemical compositions of the test solution and groundwater before the onset of the test (pretest) during the ethanol-amended push-pull test.

		Test Solution	Pretest
Volume	(gal)	100	n.a.
T	(°C)	16.3	15.6
pH		7.3	7.6
Conductivity	(μS)	1960	2020
Alkalinity	(mM)	20.66	13.2
Ethanol	(mM)	40	n.a.
Bromide	(ppm)	100	n.a.
Chloride	(ppm)	23.57	322.97
Ferrous iron	(ppm)	0.03	0.95
Sulfate	(ppm)	48.67	3.53
Arsenite	(ppb)	8.8	1800
Arsenate	(ppb)	2000	340

n.a., not applicable

## CHAPTER III

### MATERIALS AND METHODS

#### 3.1. Sample Collection

Groundwater samples were collected according to USGS standard procedures (U.S. Geological Survey, 2006). Before sampling, groundwater was filtered through 0.45 micron Quickfilter FF8200T filters from QED Environmental Systems. Gas samples were collected using a passive sampling procedure (Spalding and Watson, 2006). Water levels inside and outside of the packers was recorded before the sampling using a water level meter (Waterline Envirotech, Ltd).

Groundwater samples for trace metal analysis were preserved using trace metal grade nitric acid (0.5 ml into filled 60 ml polyethylene bottles). Samples for arsenic analysis were preserved using 250 mmolal EDTA (100 $\mu$ l into 30ml filled opaque polyethylene bottles) or trace metal grade HCl (130 $\mu$ l into 30ml filled bottles).

To examine the minerals of the aquifer matrix during the experiment, packets of aquifer materials were incubated inside the packers. Fresh Fisher formation rocks were retrieved from a road cut near the well site; to assure minimal weathering alterations, no exposed rock was collected. The rock samples were then crushed to particles with diameters <149  $\mu$ m, 149 - 420  $\mu$ m and 420 - 700  $\mu$ m. The rock particles of <149  $\mu$ m, 149 - 420  $\mu$ m and 420 - 700  $\mu$ m were then mixed in the volume ratio of



2:1:1 and transferred into sewn plastic screen packets. These sediment packets were deployed in the test well for at least 3 months prior to the push-pull tests to equilibrate with the groundwater environment. The mineral samples were retrieved every week during the ethanol-amended experiment.

### 3.2. Chemical Analysis

Unstable chemical parameters were analyzed in the field during the sampling. pH and temperature were analyzed using standard electrodes. Sulfide and ferrous iron were analyzed using Hach method 2244500 and 103769 on a Hach DR 2000 spectrophotometer. Alkalinity was analyzed immediately following the sampling using the standard buret titration method and results calculated using the USGS Alkalinity Calculator (U.S. Geological Survey, 2009).

Gas samples were analyzed for methane and carbon dioxide using SRI gas chromatography. Water samples were analyzed for arsenic and anions. The anions were analyzed using a Dionex ion chromatographer. Arsenic co-precipitated with minerals was analyzed using the arsenic sequential extraction procedure by Keon *et al.* (2001). Iron minerals were analyzed using total “reactive” Fe extraction procedure and a poorly crystalline Fe(III) and surface-associated Fe(II) extraction procedure, as outlined by Eric Roden (personal communication, see Appendix A). Sulfide minerals were analyzed using a sequential extraction procedure described by M. Kirk (personal communication, see Appendix A), determining acid volatile sulfide and chromium reducible sulfide.

### 3.3 Data Analysis

#### 3.3.1 Water Samples

The mixing fraction,  $f_t$ , the relative amount of the groundwater mixed into the test solution around the well borehole, is defined as,

$$f_t = \frac{M_{tr(t)} - M_{tr,bk}}{M_{tr,test} - M_{tr,bk}} \quad (\text{eq. 3})$$

where  $M_{tr(t)}$  is the concentration of tracer in the sample collected at time  $t$ ,  $M_{tr,bk}$  is the concentration of the tracer in the groundwater (background), and  $M_{tr,test}$  is the tracer concentration of the test solution. The control experiment used chloride ion,  $\text{Cl}^-$ , as the tracer; the ethanol-amended experiment used bromide ion,  $\text{Br}^-$ , as the tracer.

The mixing fraction,  $f_t$ , quantifies the changes in chemical concentrations due to groundwater mixing. To do so, this mixing fraction is applied to chemical species (i.e.  $\text{SO}_4^{--}$ ,  $\text{Fe}^{++}$ , etc.) to calculate dilution adjusted concentrations. Taking sulfate concentration as an example,

$$\Delta M_{\text{SO}_4^{--}} = M_{\text{SO}_4^{--},bk} + f_t \times [M_{\text{SO}_4^{--},test} - M_{\text{SO}_4^{--},bk}] \quad (\text{eq. 4})$$

where  $\Delta M_{\text{SO}_4^{--}}$  is the concentration of sulfate if only groundwater mixing occurs in the aquifer,  $f_t$  is the mixing fraction at time  $t$ ,  $M_{\text{SO}_4^{--},test}$  is the concentration of sulfate in the test solution, and  $M_{\text{SO}_4^{--},bk}$  is the groundwater sulfate concentration. The difference between  $\Delta M_{\text{SO}_4^{--}}$  and  $M_{\text{SO}_4^{--},t}$ , where  $M_{\text{SO}_4^{--},t}$  is the concentration of sulfate at time  $t$ , is the concentration of sulfate introduced or removed by microbial metabolisms and chemical reactions.

### 3.3.2. Solid Samples

Arsenic, iron, and sulfide sequential extraction data were all normalized by the weight of samples. Taking arsenic concentrations as an example, the concentrations ( $\mu\text{g}/\text{g}_{\text{sed}}$ ) of arsenic in solid-phase were calculated by,

$$As = \frac{r_{(ppb)} \times V_{(L)}}{g_{sed}} \quad (\text{eq. 5})$$

where  $r$  is the concentration in ppb, in the extracted solution,  $v$  is the volume of the solution, in Liters, and  $g_{\text{sed}}$  is the mass of sediment extracted. Iron and sulfide data were processed similarly.

## CHAPTER IV

### RESULTS

#### 4.1. Depth Interval of Experiments

The depth interval for the push-pull tests are selected by conducting aquifer slug tests. Figure 3 illustrates how the transmissivity and connectivity of the bedrock aquifer around the well borehole vary with the depth of the borehole. At the interval 95 feet below the ground, the transmissivity is large, about 200 mL/m, but the connectivity is small, about 10%. Also, at the depth interval 125 ft below the ground, the transmissivity is large, >250 mL/m, and the connectivity is near zero. The depth interval of 95 ft was selected for the push-pull experiments.

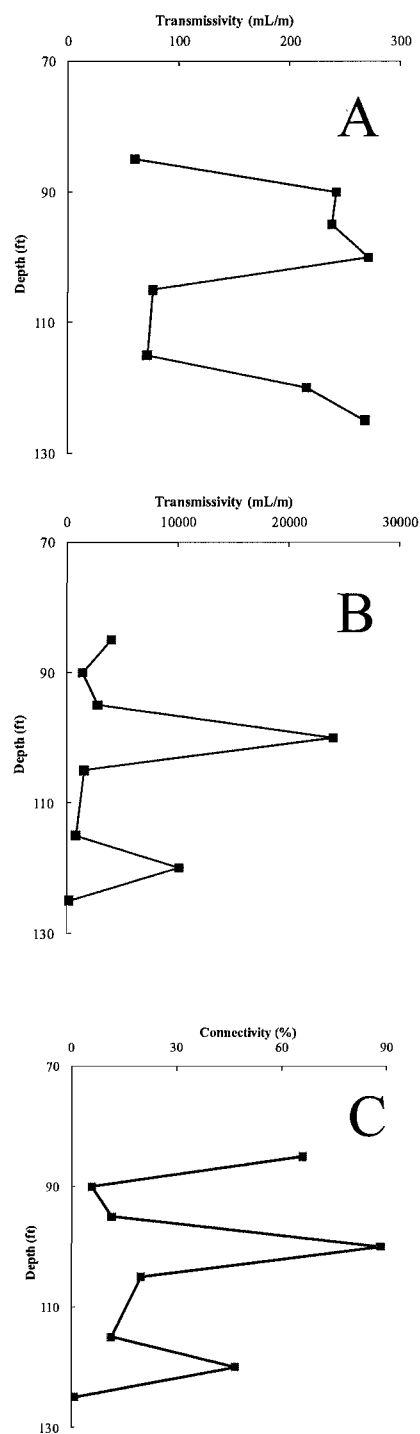


FIGURE 3. Changes in the transmissivity of the depth interval isolated by the packers (A), outside of the packers (B), and connectivity (C) with the depth of the well borehole.

#### 4.2. Control Experiment

During the experiment, the injection of the test solution displaced groundwater laterally from the well borehole. The groundwater within the perforated zone of the packer was sampled periodically to monitor the progress of the experiment.

Groundwater temperature remained relatively stable during the experiment (Fig. 4).

Groundwater alkalinity also remained relatively constant, ranging from 10 to 15 meq/L (Fig. 4). Groundwater pH was 6.8 at the beginning of the experiment and gradually increased, ending at a pH of 7.6 (Fig. 4). After an initial increase from 1500  $\mu$ S to 1800  $\mu$ S, the groundwater conductivity remained relatively constant over the duration of the experiment (Fig. 4).

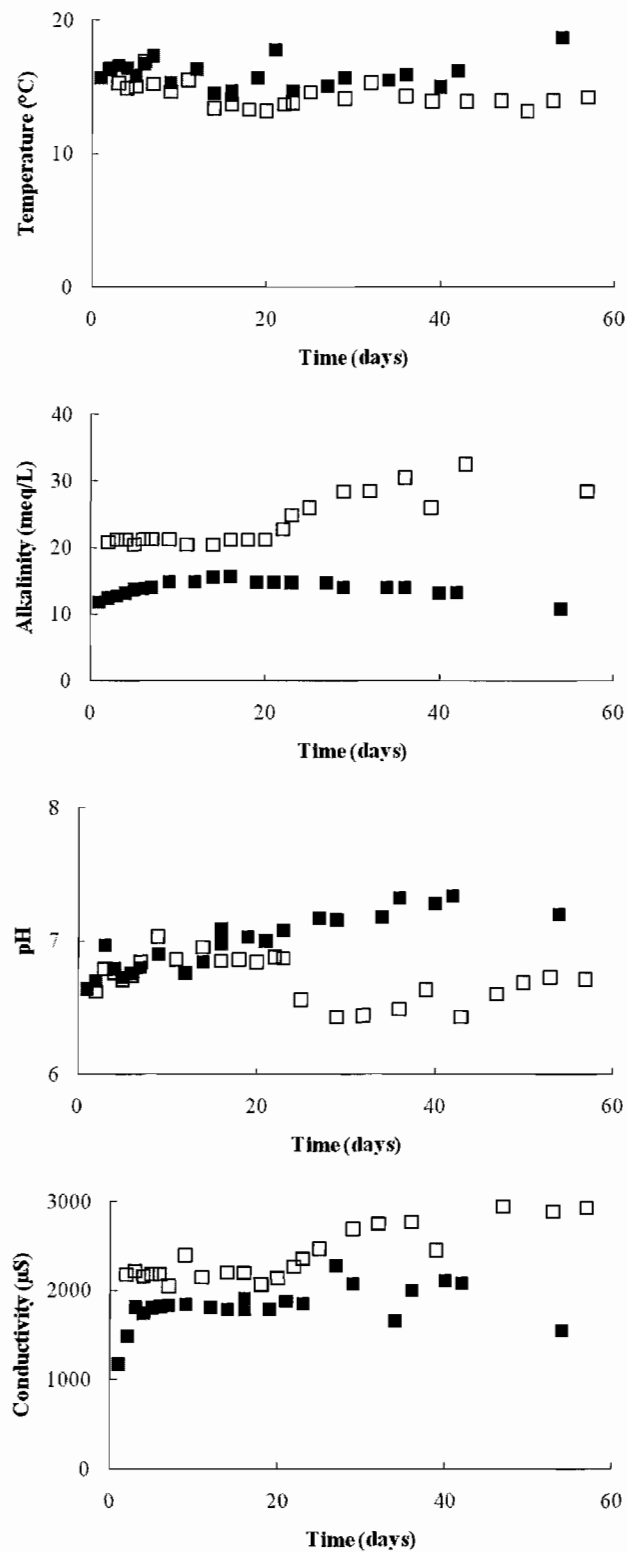


FIGURE 4. Changes with time in temp ( $^{\circ}\text{C}$ ), alkalinity (meq/L), pH, and conductivity ( $\mu\text{S}$ ) during the control(■) and ethanol-amended(□) experiments.

As the experiment progressed, the concentrations of most chemical species varied. For example, chloride concentration remained constant at around 200 ppm until day 18. At day 18, there was sharp increase in the concentration of chloride, followed by a gradual increase from day 20 to the end of the experiment (Fig. 5).

Arsenate decreased as the experiment progressed. Arsenite concentrations, on the other hand, increased with time (Fig. 6). Organic arsenic species, the product of microbial arsenic detoxification, also occurred in the groundwater. Methylarsonate (MMA) concentrations decreased with the experiment progress, while dimethylarsinite (DMA) concentrations showed the opposite trend. Sulfate concentration increased gradually from about 100 ppm at the beginning of the experiment to 220 ppm at the end (Fig. 7). Sulfide, ferrous iron, and methane concentrations remained close to zero throughout the duration of the experiment (Figs. 7-9).

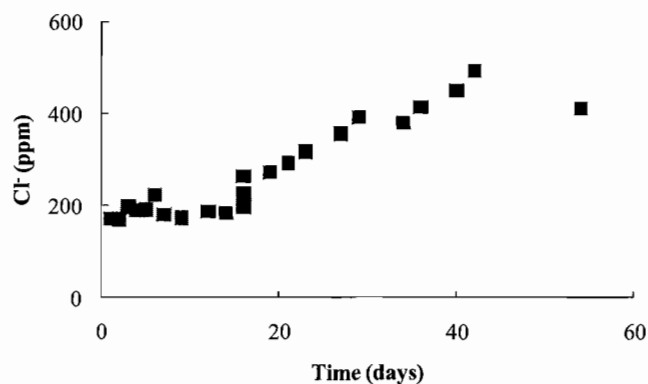


FIGURE 5. Changes with time in the concentration of chloride ( $\text{Cl}^-$ ) during the control experiment.



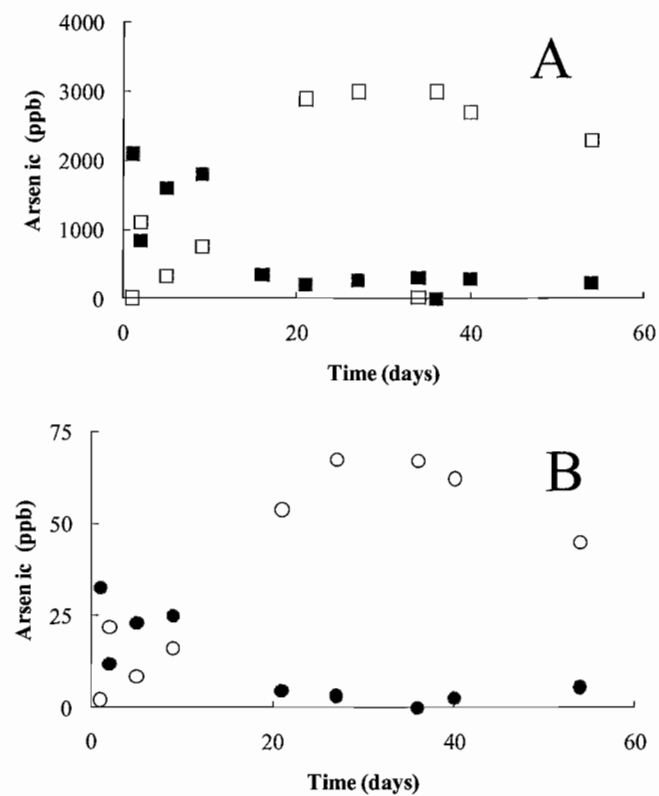


FIGURE 6. Changes with time in the concentrations of arsenate (■) and arsenite (□) (panel A), and methylarsonate (MMA, ●), and dimethylarsinite (DMA, ○) (panel B) during the control experiment.

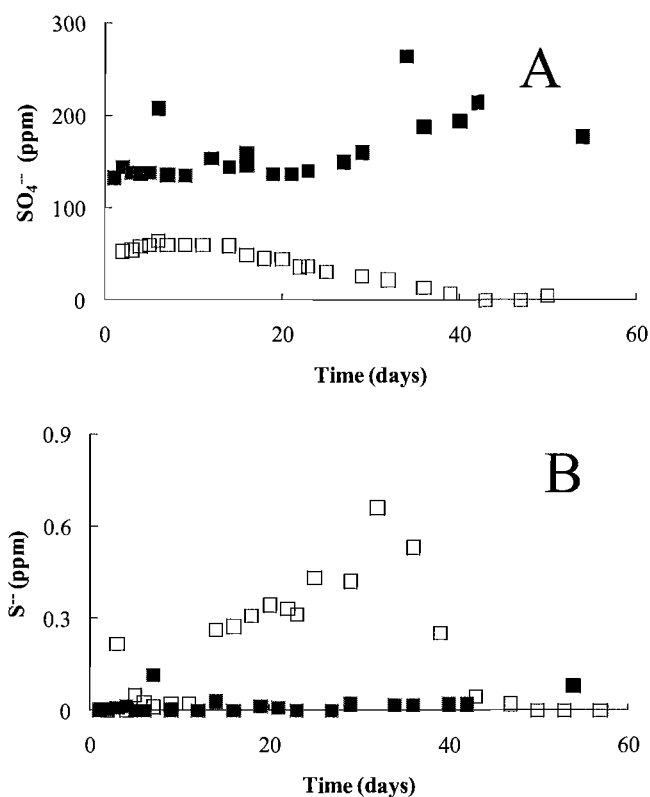


FIGURE 7. Changes with time in sulfate (panel A) and sulfide (panel B) concentrations during the control (■) and ethanol-amendment experiments (□).

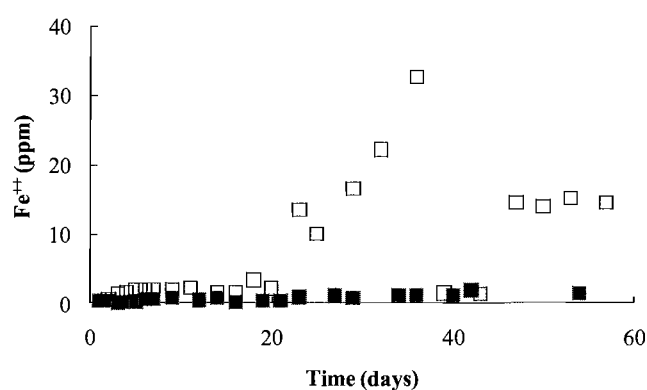


FIGURE 8. Changes with time in ferrous iron concentrations during the control (■) and ethanol-amended (□) experiments.

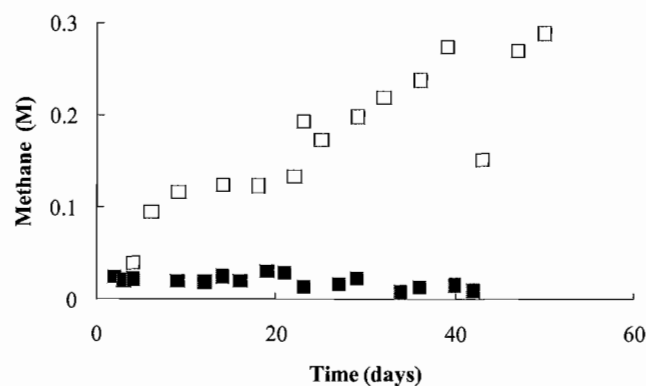


FIGURE 9. Changes with time in methane concentrations during the control (■) and ethanol-amendment experiments (□).

#### 4.2.1. Reaction Kinetics

The changes in the concentrations of chemical species resulted from multiple processes, including groundwater mixing, geochemical reactions, and microbial metabolism. To demonstrate the impact of groundwater mixing, Figure 10 shows the changes with time in the mixing fraction,  $f$ , during the control experiment. Because chloride is an inert chemical species and does not react significantly with the chemical species in groundwater or aquifer matrix, the mixing fraction is calculated based on the changes with time in  $\text{Cl}^-$  concentrations (Fig. 5). The mixing fraction remained near unity during the first 18 days of the experiment (Fig. 10). In other words, there was a delay of about 18 days before the onset of the groundwater mixing. After day 18, the mixing fraction decreased linearly with time, indicating a continuous mixing of the groundwater into the test solution.

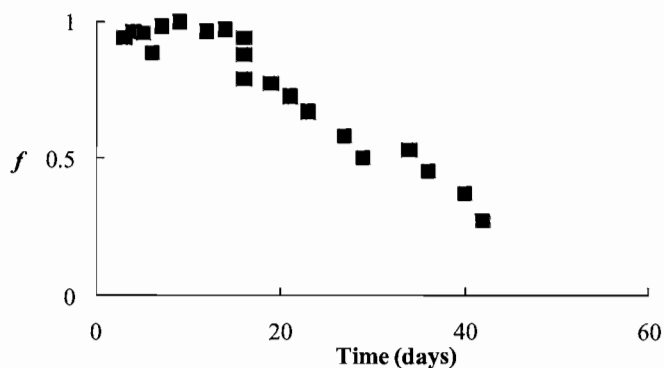


FIGURE 10. The changes in the mixing fraction ( $f$ ), calculated based on  $\text{Cl}^-$  concentrations, during the control experiment.

The impact of geochemical reactions and microbial metabolism on chemical species of interest can be evaluated based on the mixing fraction (Eq. 3) and the observed temporal changes in the concentrations. For example, based on arsenate concentrations observed after 20 days into the experiment (Fig. 11), arsenate accumulated in the groundwater at a rate of 32.98 ppb/day and arsenite concentrations decreased at a rate of 48.98 ppb/day (Fig. 11). As a result, the total arsenic concentrations, the sum of arsenate and arsenite concentrations, decreased at a rate of 16.026 ppb/day (Fig. 11).

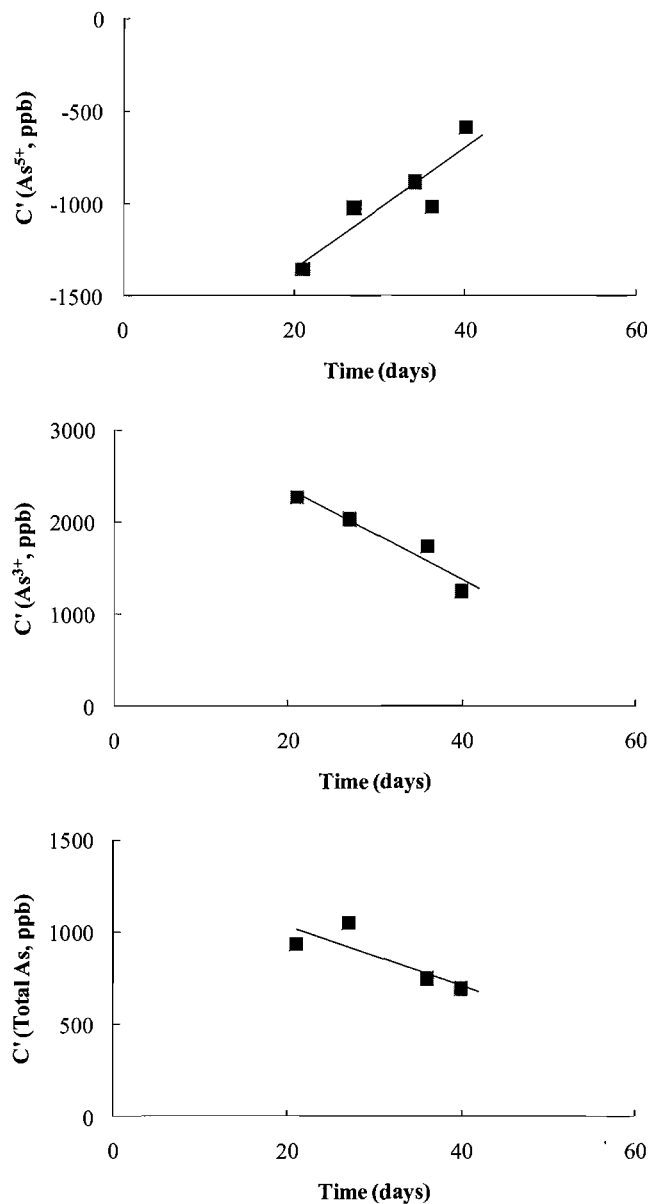


FIGURE 11. Dilution adjusted arsenate, arsenite, total arsenic concentration ( $C'$ ) during the control experiment. Data points are calculated from measured concentrations. Lines represent the best fit to the data points (arsenate,  $y = 32.978x - 2013.3$ ,  $R^2 = 0.8034$ ), (arsenite,  $y = -48.98x + 3343.3$ ,  $R^2 = 0.9231$ ), and (total As,  $y = -16.026x + 1355.7$ ,  $R^2 = 0.6998$ ).

Based on fluoride concentrations after 20 days into the experiment, fluoride was produced at a rate of 0.2337 ppm/day (Fig. 12). Comparatively, sulfate

concentrations also increased during the experiment at a rate of 1.4585 ppm/day (Fig. 12). Based on the changes in sulfide concentrations (Fig. 12), sulfide was produced at a rate of 0.0008 ppm/day. In addition, ferrous iron concentrations steadily increased as the groundwater mixing occurred at a small rate of 0.0367 ppm/day (Fig. 13). Comparatively, methane concentrations gradually decrease with time at a rate of 0.0008 molal/day (Fig. 13).

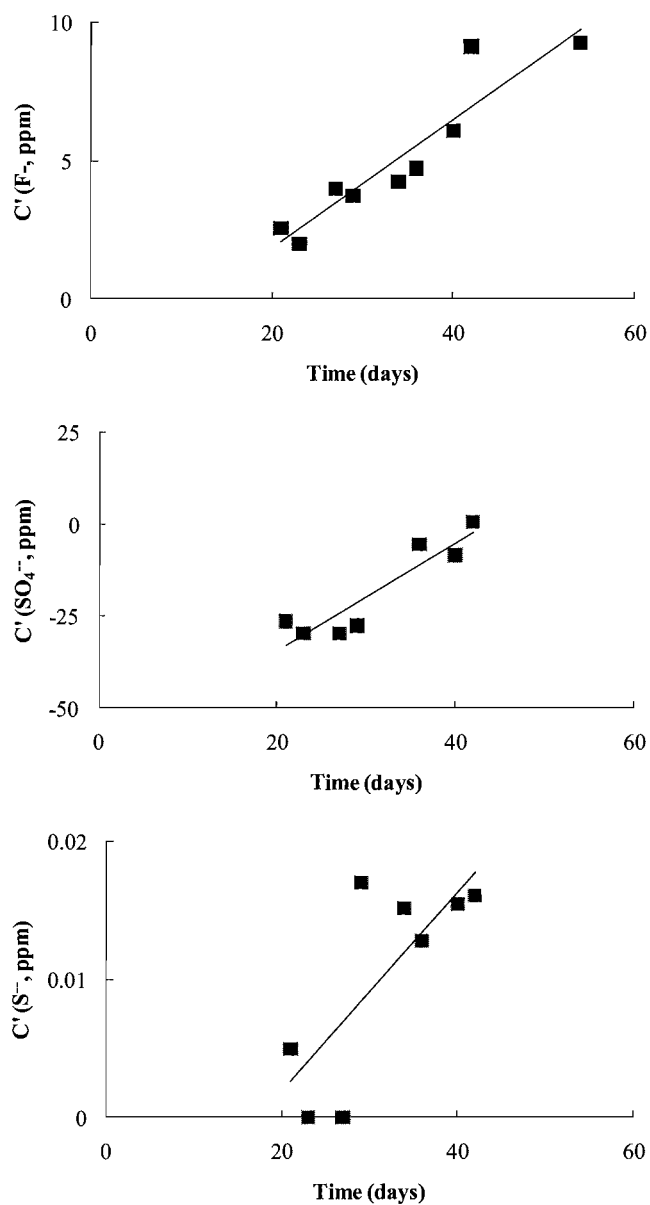


FIGURE 12. Dilution adjusted fluoride, sulfate, and sulfide concentrations ( $C'$ ) during the control experiment. Data points are calculated from measured concentrations. Lines represent the best fit to data points (fluoride,  $y = 0.2337x - 2.8603$ ,  $R^2 = 0.865$ ) (sulfate,  $y = 1.4585x - 63.451$ ,  $R^2 = 0.8444$ ), (sulfide,  $y = 0.0008x - 0.0168$ ,  $R^2 = 0.5354$ ).

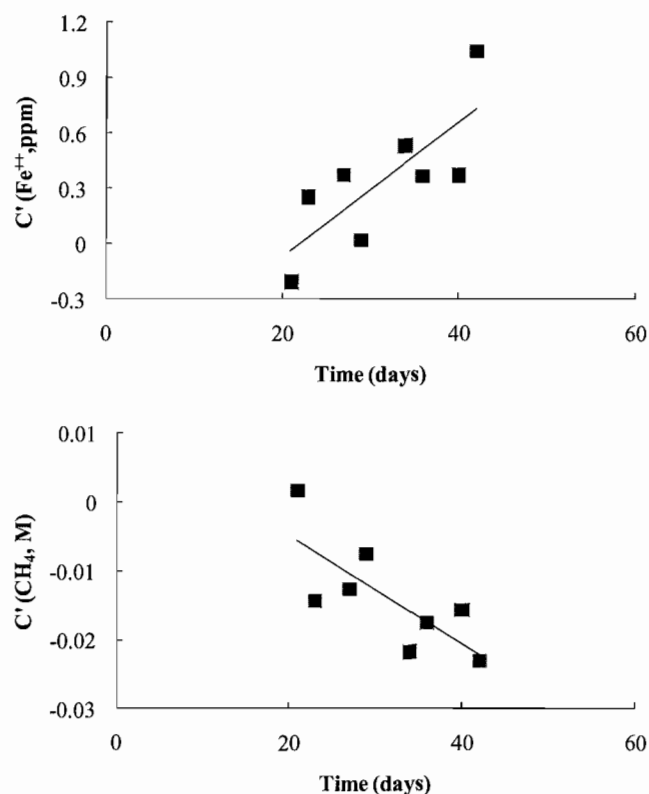


FIGURE 13. Dilution adjusted ferrous iron and methane concentrations ( $C'$ ) during the control experiment. Data points are calculated from measured concentrations. Lines represent the best fit to data points (ferrous iron,  $y = 0.0367x - 0.8098$ ,  $R^2 = 0.5981$ ) and (methane,  $y = -0.0008x + 0.0112$ ,  $R^2 = 0.5835$ ).

#### 4.3. Ethanol-Amended Experiment

During the ethanol-amended experiment, the test solution containing ethanol was injected into the aquifer to stimulate microbial metabolisms (see Table 3). Groundwater temperature remained fairly constant throughout the experiment, beginning at 16.3°C and ending at 13.2°C (Fig. 4). Alkalinity first remained roughly constant, but after 20 days into the experiment, alkalinity increased gradually with time to 32 meq/L (Fig. 4). Groundwater pH was 6.62 at the beginning and decreased to 6.4 at day 34, but then it reversed and increased to 7.03 at the end of the experiment



(Fig. 4). Conductivity began at 2180  $\mu\text{S}$  and remained constant until day 20. After day 20, conductivity began to rise to an ending value of 3000  $\mu\text{S}$  (Fig. 4).

Figure 14 shows how the concentrations of bromide,  $\text{Br}^-$ , varied as the experiment progressed. Bromide was added to the test solution as the groundwater tracer and the concentration at the beginning of the experiment was 100 ppm. The concentrations decreased as the experiment progressed. At day 18, there was sharp decrease in the concentration. At the end of the experiment, bromide concentrations decreased to about 50 ppm.

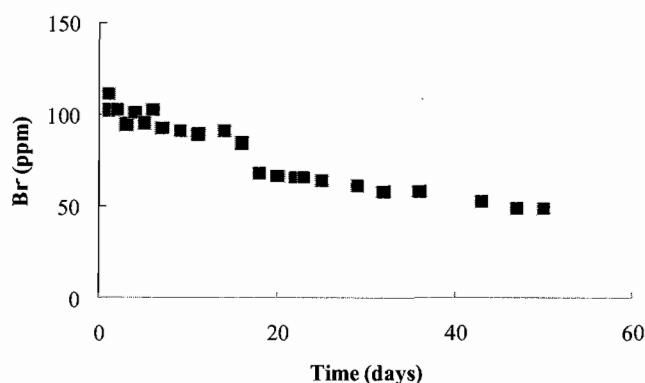


FIGURE 14. Changes with time in bromide concentration during the ethanol-amended experiment.

As shown in Figure 15, during the first two days of the experiment, arsenate was consumed while arsenite accumulated. Concurrently, MMA concentrations decreased while DMA concentrations increased. From day two to 15, the concentrations of arsenate and arsenite remained relatively small, less than 300 ppb. Arsenate concentrations decreased to near zero. At day 18, arsenite concentration increased sharply to 2200 ppb and then started to decrease. After 38 days into the experiment, arsenite began to increase with time, and reached 1400 ppb at day 53. By

day 10, DMA concentrations began to drop to about 5 ppb and MMA concentrations remained near zero. After 30 days into the experiment, DMA concentrations started to increase and increased to 35 ppb at the end of the experiment.

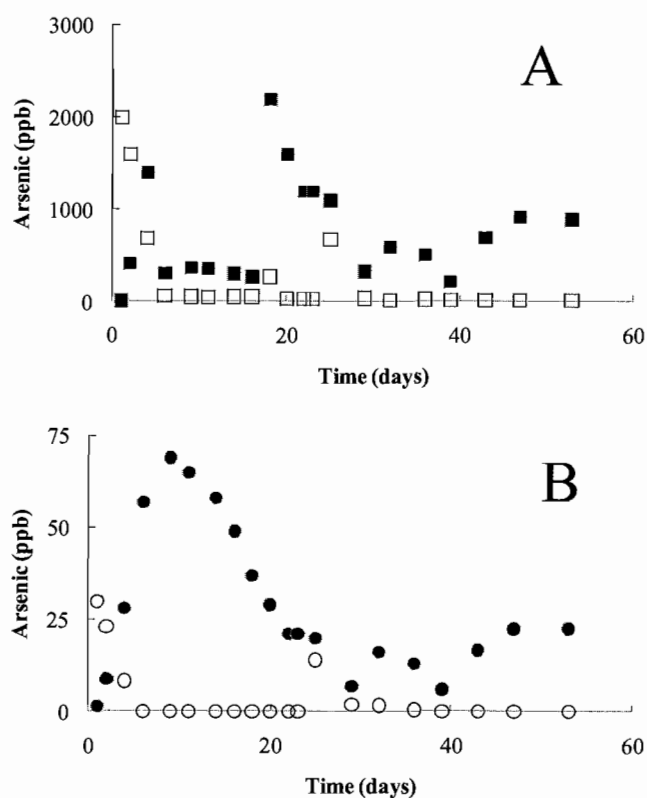


FIGURE 15. Changes with time in the concentrations of arsenate (□) and arsenite (■) (panel A), and MMA (○) and DMA (●) (panel B) during the ethanol-amended experiment.

During the first 15 days of the experiment, both sulfide and ferrous iron concentrations remained relatively small (Figs. 7 and 8). After 15 days into the experiment, however, both ferrous iron and sulfide started to accumulate. Comparing Figures 10 and 22, the accumulation of ferrous iron corresponds to the sharp increase in arsenite concentrations in the groundwater. At day 38, ferrous iron started to

decrease sharply to 3 ppm. The concentrations then increased to 15 ppm at day 43, and remained roughly constant afterwards. As shown in Figure 7, sulfide concentrations increased to a maximum value of 0.62 ppm by day 33 and then started to decline. The concentration reached zero about 50 days into the experiment.

In addition to aqueous species in the groundwater, redox chemical species (e.g., iron, sulfur, and arsenic) also occur as minerals and chemical species sorbed onto the aquifer matrix. Iron associated with the aquifer matrix was analyzed using the standard protocol of iron sequential extraction and the results are shown in Figure 16. This analysis separates iron minerals and iron sorbed onto the aquifer matrix into ferric and ferrous iron (Eric Roden, personal communication, see Appendix A). The abundance of ferrous iron associated with solid-phase shows a general upward trend before the groundwater mixing started, while the abundance of amorphous ferric iron minerals remained roughly constant during the experiment (Fig. 16).

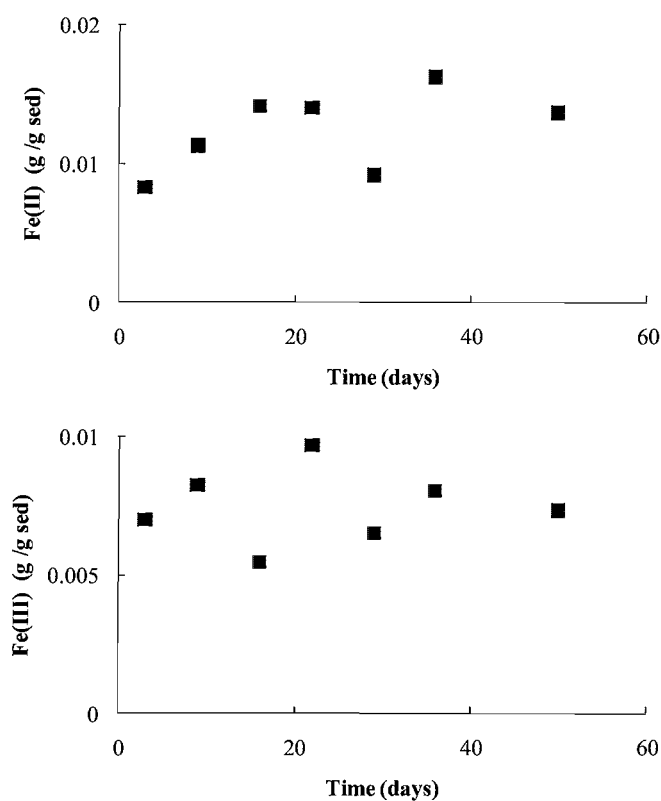


FIGURE 16. The changes in the amount of solid-phase ferrous iron Fe(II) and solid-phase amorphous ferric iron Fe(III) during the ethanol-amended experiment.

Figure 17 show the results of sulfide mineral extraction analysis. This approach analyzes acid volatile sulfide minerals (AVS), such as amorphous iron sulfide, and Cr-reducible sulfide minerals (CRS), such as pyrite. As shown in Figure 17, there were little AVS and CRS in the aquifer material during the first half of the experiment. After day 35, both AVS and CRS increased significantly, corresponding to the decreases in sulfide and ferrous iron concentrations in groundwater (Figs. 7 and 8).

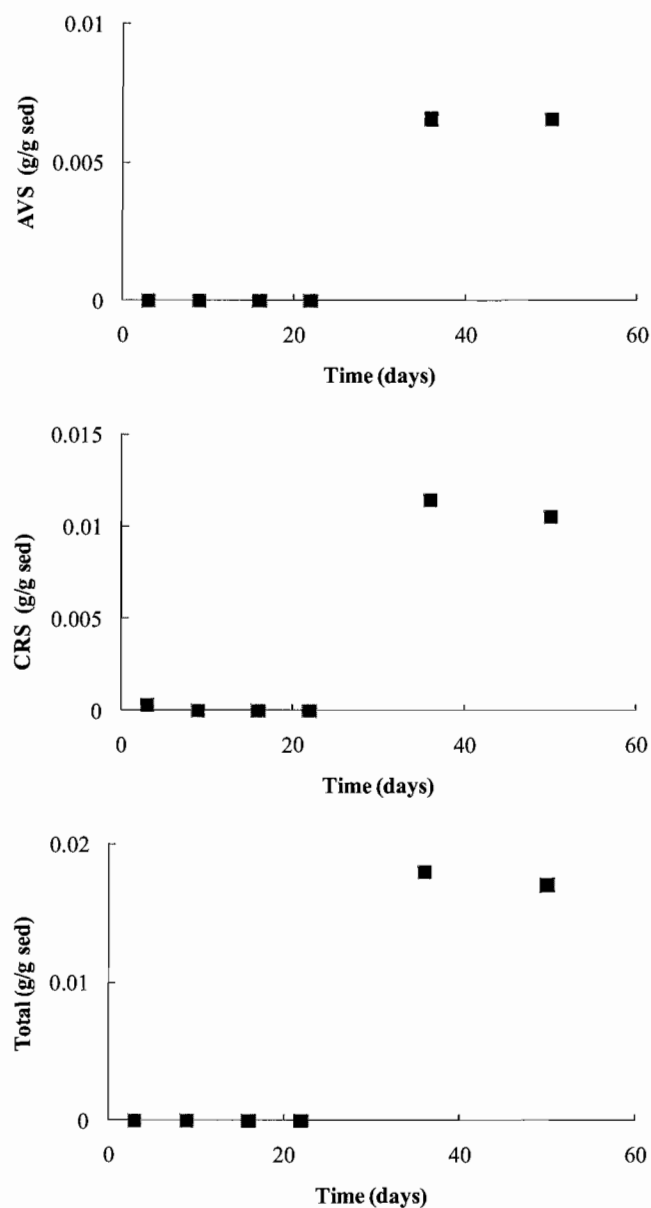


FIGURE 17. Changes in the abundance of acid volatile sulfide (AVS), chromium reducible sulfide (CRS), and total solid-phase sulfide over the duration of the ethanol-amended experiment.

Figures 18-21 show the changes with time in the concentrations of arsenic associated with solid phases of the aquifer. The arsenic sequential extraction differentiates arsenic bound to aquifer matrix into six pools, such as ionically-bound,

co-precipitated with Fe oxyhydroxides, co-precipitated with acid-volatile sulfide (AVS), associated with amorphous Fe oxyhydroxides, etc. (Keon *et al*, 2001). The ionically-bound concentration increased at the beginning of the experiment, and peaked at day 16, the time where the concentrations of ferrous iron and sulfide accumulated (Fig. 18). Arsenic co-precipitated with AVS increased gradually as the experiment progressed; the concentrations of arsenic associated with amorphous iron minerals experienced their low values at day 16 and steadily increase from day 16 (Fig. 19). The concentration of amorphous  $\text{As}_2\text{S}_3$  increased and reached its maximum value at day 29 (Fig. 20). The concentrations of strongly-adsorbed arsenic, arsenic co-precipitated with crystalline iron oxyhydroxides remained relatively constant as the experiment progressed. Figure 21 shows the changes in total soil-bound arsenic over the duration of the ethanol-amended experiment.

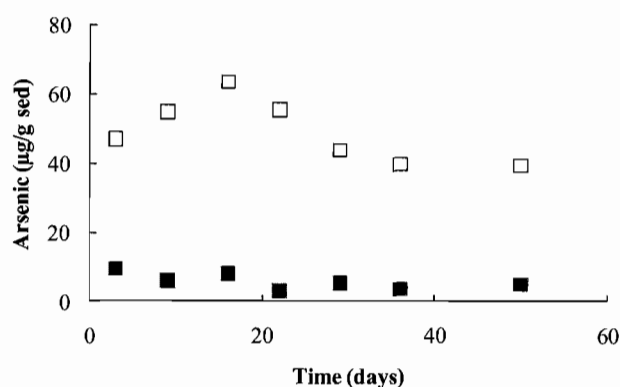


FIGURE 18. Changes in the concentrations of ionically-bound (■) and strongly-adsorbed arsenic (□) over the duration of the ethanol-amended experiment.

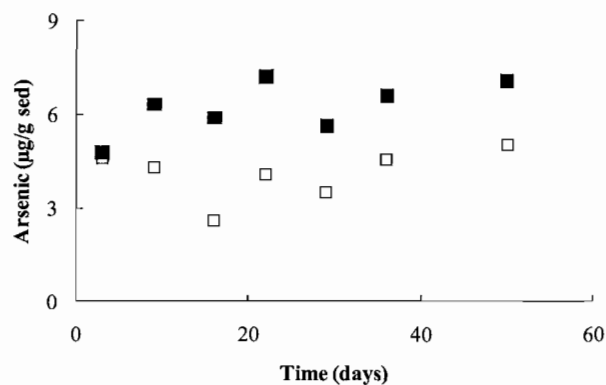


FIGURE 19. Changes in the concentrations of arsenic coprecipitated with AVS (■) and arsenic-rich amorphous iron oxyhydroxides (□) over the duration of the ethanol-amended experiment.

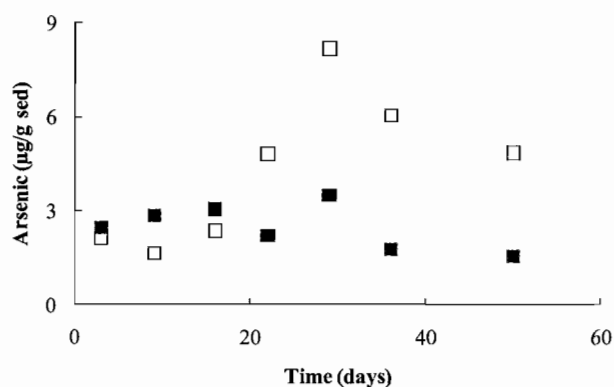


FIGURE 20. Changes in the concentrations of arsenic coprecipitated with crystalline iron oxyhydroxides (■) and amorphous  $\text{As}_2\text{S}_3$  (□) over the duration of the ethanol-amended experiment.

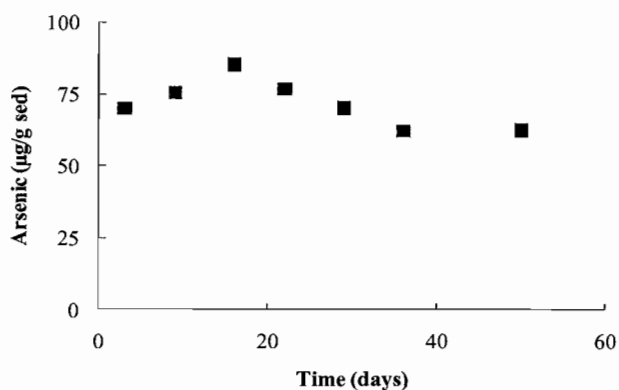


FIGURE 21. Changes in the concentration of total solid-bound arsenic over the duration of the ethanol-amended experiment.

#### 4.3.1. Reaction Kinetics

The ethanol experiment produced redox reactions modified by groundwater mixing. Changes in the aqueous and solid-phase redox species suggest that ethanol-amendment stimulated microbial iron and sulfate reduction. The progress of groundwater mixing is quantified based on the changes with time in bromide concentrations and expressed as the mixing fraction,  $f$ , (Fig. 22). The change in the value of  $f$  during the experiment indicates that the groundwater continuously mixed into the test solution around the perforated zone in the well borehole. Notably, bromide concentrations decreased sharply at day 18 (Fig. 14), indicating an influx of the groundwater due a storm event changing local hydrological conditions.

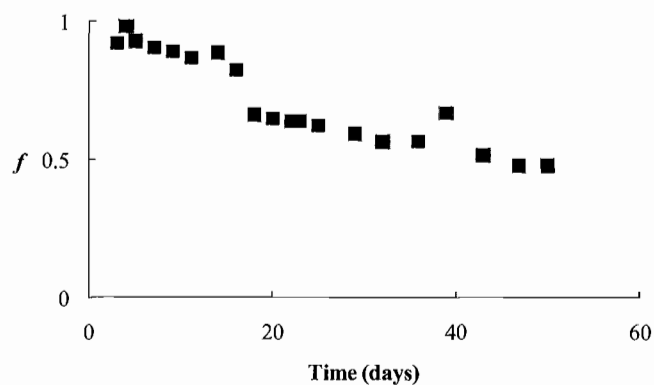


FIGURE 22. The changes in the mixing fraction ( $f$ ), determined based on  $\text{Br}^-$  concentrations, during the ethanol-amended experiment.

The rates of the changes in the concentrations of chemical species can be evaluated based on the mixing fraction (Eq. 3) and the observed temporal changes in the concentrations. For example, arsenate concentrations observed after 2 weeks (Fig. 23), increased steadily at a rate of 6.429 ppb/day, while arsenite concentrations decreased at a rate of 27.743 ppb/day (Fig. 23). As a result, total arsenic, the sum of



arsenate and arsenite concentrations, decreased at a rate of 22.3 ppb/day as the experiment progressed (Fig. 23).

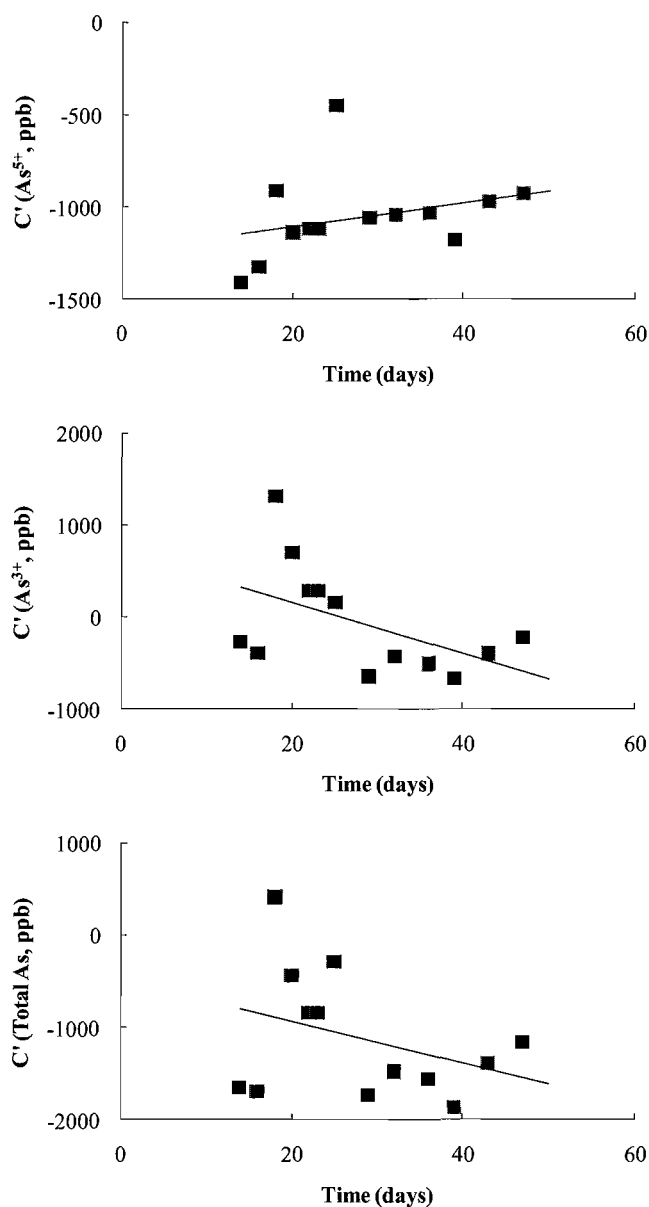


FIGURE 23. Changes in the dilution adjusted arsenate, arsenite, and total arsenic concentrations ( $C'$ ) during the ethanol-amended experiment. Data points are calculated from measured concentrations. Lines represent the best fit to data points (arsenate,  $y = 6.4286x + 1230.1$ ,  $R^2 = 0.0883$ ) (arsenite,  $y = -27.743x + 723.78$ ,  $R^2 = 0.2564$ ), and (total arsenic,  $y = -22.38x - 484.19$ ,  $R^2 = 0.1217$ ).

After 20 days into the experiment fluoride was produced at a rate of 117.49ppm ln(days) (Fig. 24). Comparatively, sulfate consumption occurred at a rate of 1.1983 ppm/day (Fig. 24). Potential mechanisms accounting for the consumption of sulfate include sulfate mineral precipitation and sulfate reduction to sulfide. Because of the low concentrations of cations, including those of barium, the precipitation of sulfate minerals during the test was highly unlikely. Considering sulfide concentrations increased during the experiment, sulfate is assumed to be reduced to sulfide; sulfide concentrations increased from day 14 to day 32 (Fig. 24). The rate of sulfide production, calculated based on the groundwater mixing and sulfide concentrations between day 14 and 32 of the experiment, is 0.0182 ppm/day (Fig. 24). In addition, ferrous iron concentrations increased as groundwater mixing occurred at a rate of 1.3897 ppm/day (Fig. 25).

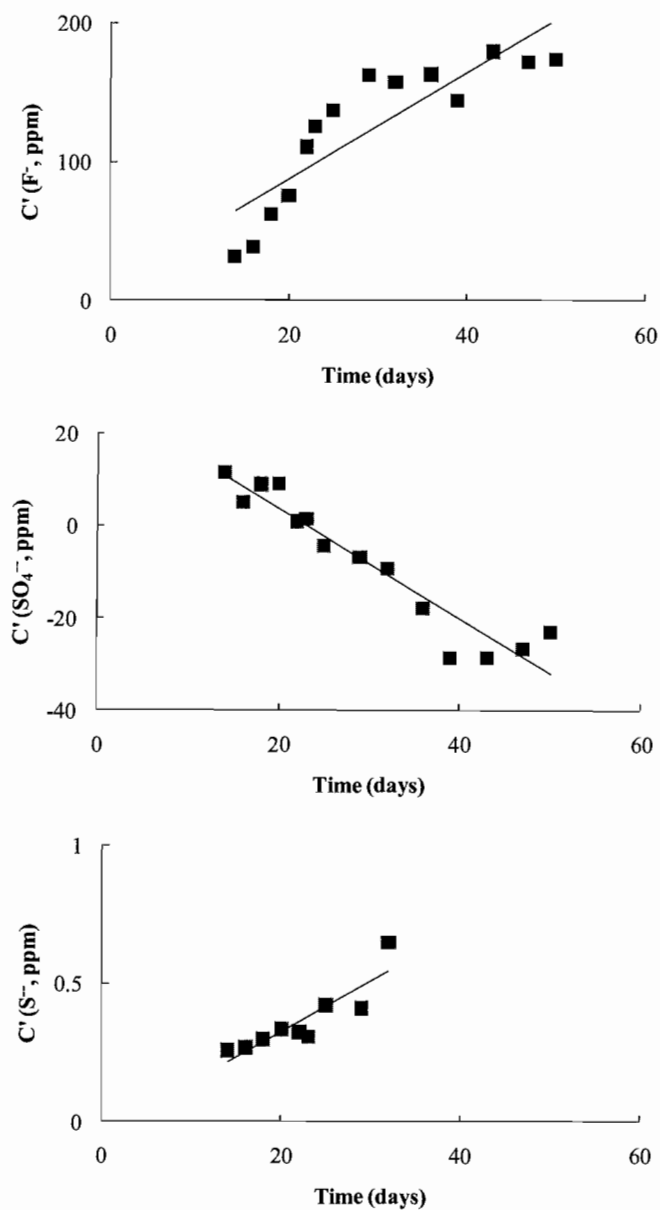


FIGURE 24. Changes in the dilution adjusted fluoride, sulfate, and sulfide concentrations (C') during the ethanol-amended experiment. Data points are calculated from measured concentrations. Lines represent the best fit to data points (fluoride,  $y = 3.8146x + 11.384$ ,  $R^2 = 0.7582$ ) (sulfate,  $y = -1.1983x + 27.778$ ,  $R^2 = 0.9069$ ), and (sulfide,  $y = 0.0182x - 0.0373$ ,  $R^2 = 0.7742$ ).

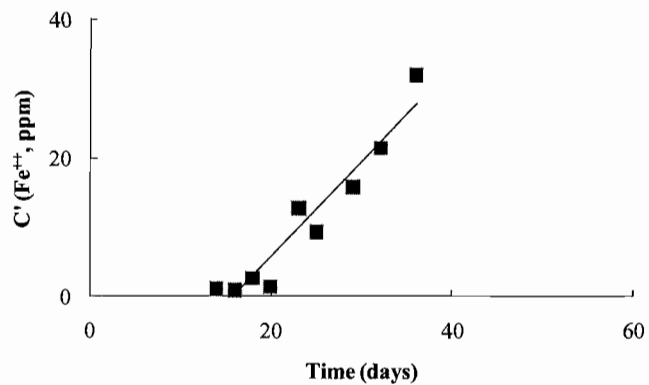


FIGURE 25. Changes in the dilution adjusted ferrous iron concentrations ( $C'$ ) during the ethanol-amended experiment. Data points are calculated from measured concentrations. Line represents the best fit to data points ( $y = 1.3897x - 22.035$ ,  $R^2 = 0.9195$ ).

## CHAPTER V

### DISCUSSION

Two push-pull tests, a control test followed by an ethanol-amended test, were performed to quantify *in situ* rates of microbial arsenate reduction, iron reduction, and sulfate reduction. Microbial metabolisms oxidize electron donors (e.g., ethanol), reduce electron acceptors (e.g. ferric iron, sulfate, bicarbonate, etc.), accumulating ferrous iron, sulfide, methane, and other end products in the aquifer. The rates of microbial metabolism are calculated based on the changes in the chemical composition of groundwater samples collected during the experiments.

The rates of microbial iron reduction, sulfate reduction, and arsenate reduction in the control experiment as compared to those observed in the ethanol-amended experiments are compared in Table 4. The largest rate observed during the control experiment was that of sulfate production, at 1.4585 ppm/day; the smallest rate was that of sulfide production, at only 0.0008 ppm/day. In other words, microbial metabolisms, such as sulfate reduction, are relatively insignificant. The rates observed during the control experiment represent those under *in situ* conditions because no electron donors were added into the aquifer and because the test solution was synthesized using the groundwater from a well close to the test site. The small rates of microbial metabolisms agree with previous observations that microbial metabolisms in

pristine aquifers are limited by the small concentrations of electron donors (Istok *et al*, 2004).

TABLE 4. Rates of chemical species production during the push-pull tests.

Microbial Metabolism	Indicative Chemical Species	Units	Rates	
			Control Test	Ethanol-Amendment Test
Iron Reduction	$\text{Fe}^{2+}$	ppm/day	0.0367	0.2863
Sulfate Reduction	$\text{SO}_4^{--}$	ppm/day	1.4585	-0.4027
	$\text{HS}^-$	ppm/day	0.0008	0.0074
Arsenate Reduction	$\text{As}^{5+}$	ppb/day	32.9780	6.4286
	$\text{As}^{3+}$	ppb/day	-48.980	-27.743
	$\text{F}^*$	ppm/day	0.2337	3.8146

\* Fluoride concentration likely indicates dissolution of iron minerals, iron oxyhydroxides, or phyllosilicates during microbial iron reduction.

The injection of ethanol during the ethanol-amendment experiment significantly stimulated the metabolism of microorganisms in the aquifer. Microorganisms are capable of oxidizing ethanol to bicarbonate, increasing groundwater alkalinity. At the same time, the degradation of ethanol produces protons, decreasing groundwater pH. As shown in Figure 4, alkalinity increased and groundwater pH decreased simultaneously about 20 days into the experiment.

Compared to those in the control experiment, the rates of different microbial metabolisms in the ethanol-amended experiment increase by a factor of 10 (see Table 4). For example, the rate of iron reduction is 0.2863 ppm/day in the ethanol-amended experiment, but only 0.0367 ppm/day in the control experiment. Microbial iron

reduction dissolves ferric iron minerals, such as goethite and iron-containing phyllosilicates. As a result, ions in these minerals are released into groundwater. For example, fluoride, a relatively inert chemical species, accumulated significantly during the ethanol-amended experiment. The rate of fluoride accumulation was 3.8146 ppm/day. Note that fluoride concentrations increased relatively slowly in the control experiment at a rate of only 0.2337 ppm/day.

Another significant microbial metabolism is sulfate reduction. The rate of sulfate reduction is 0.4027 ppm/day in the ethanol-amended experiment; whereas in the control experiment sulfate accumulated and, the rate of the accumulation is 1.4585 ppm/day (Table 4). Sulfate reduction releases sulfide; a portion of this sulfide accumulates in groundwater; the other portion is precipitated with ferrous iron and arsenite as iron sulfide and arsenic sulfide mineral. In the ethanol-amended experiment, the rate of sulfide production is 0.0074 ppm/day, much smaller than the rates of sulfate reduction. The discrepancy in rate between sulfate reduction and sulfide production results from the precipitation of ferrous iron and arsenic sulfides (Fig. 16 and 17).

The changes with time in the concentrations of redox species in groundwater and precipitated with the aquifer matrix demonstrate the close interactions between microbial metabolism and the aquifer and the interactions among different microorganisms. The ethanol-amended experiment can be divided into two stages. During the first two weeks, the change in arsenic concentrations resulted mainly from microbial arsenate reduction to arsenite and arsenite sorption. The rate of arsenate

reduction is 394.22 ppb/day and microbial arsenate reduction decreased arsenate to negligible levels, i.e. 20 ppb (Fig. 13). Arsenate reduction produces arsenite, which is then sorbed strongly onto the aquifer matrix (Fig. 18); the rate of arsenite production is 468.2 ppb/day (Fig. 13).

After two weeks into the experiment a storm caused an influx of arsenic-rich groundwater to surround the well borehole, causing arsenite concentrations to increase dramatically to more than 2000 ppb. At this time, microbial sulfate reduction and iron reduction also occurred, increasing sulfide concentrations above 0.3 ppm and ferrous iron concentrations to 32 ppm (Figs. 7 and 8). As a result, amorphous arsenic sulfide ( $\text{As}_2\text{S}_3$ ) and iron sulfide ( $\text{FeS}_2$ ) became supersaturated and precipitated out of groundwater (Figs. 17 and 20), decreasing arsenite and ferrous iron concentrations (Figs. 8 and 15). This observation was supported using Geochemist's Workbench and the thermo.dat database where many iron sulfide minerals, including pyrite and pyrrhotite, were shown to be supersaturated given the temperature, pH, and ion concentrations.

The simultaneous formation of iron sulfide and arsenic sulfide minerals suggest that ferrous iron and arsenite compete for sulfide. The sequential sulfide extraction procedure analyzes both acid-volatile sulfide and chromium-reducible sulfide. Iron sulfide is acid-volatile while arsenite sulfide is chromium-reducible. As shown in Figure 17, similar quantities of AVS and CRS are precipitated.

Forty days into the experiment, sulfate concentrations had decreased to near zero, decreasing the rates of microbial sulfate reduction to near zero. Thus, sulfide



production was not sufficient to remove arsenite that is introduced by the continuous groundwater mixing. As a result, arsenite concentrations after 40 days into the experiment began to increase (Fig. 15).

Comparing the observations of the control and ethanol-amendment experiments (Figs. 4-25), we see that ethanol amendment stimulated a series of microbial metabolisms in the aquifer, including microbial arsenate reduction, iron reduction, and sulfate reduction. The occurrence of these processes, together with geochemical reactions (e.g., arsenic surface complexation and sulfide mineral precipitation), influenced the occurrence and levels of arsenic in the groundwater.

## CHAPTER VI

### CONCLUSION

Push-pull tests with the amendment of ethanol were conducted to study arsenic contamination in the bedrock aquifer of the Southern Willamette Basin, Oregon. The injection of ethanol into the aquifer stimulated simultaneously a series of microbial metabolisms, including arsenate reduction, iron reduction, and sulfate reduction. These metabolisms interact with arsenic surface complexation and the precipitation of arsenic sulfide and iron sulfide minerals, controlling the speciation and levels of arsenic in the groundwater. Results of the study show that arsenite is the dominant aqueous arsenic species in the aquifer and sulfide, the product of microbial sulfate reduction, controls the levels of arsenite. In the control experiment, no electron donor was added into the aquifer and no significant sulfate reduction occurred. Consequently, groundwater arsenite concentrations remained high. After ethanol was added into the aquifer, significant sulfide was produced by microbial sulfate reduction and, as a result, arsenite precipitated as amorphous arsenic sulfide. However, after sulfate reduction ceased, no sulfide was available to remove arsenite therefore arsenite concentrations increased as the groundwater mixing continued.

The efficiency of arsenic removal by sulfate reduction is adversely impacted by microbial iron reduction. Where ferrous iron is present, ferrous iron competed with

arsenite for sulfide, limiting the amount of sulfide available for the precipitation of arsenic sulfide minerals. *In situ* remediation strategies thus need to account for the complex reaction network of microbial iron reduction, sulfate reduction, arsenate reduction, sulfide mineral formation, and arsenic sorption.

APPENDIX A  
LABORATORY PROCEDURES

**Anion Standards for HPLC – IC**  
From PM2.5 Anion Analysis

For 1000mg/L:  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{F}^-$ ,  $\text{HPO}_4^{2-}$ ,  $\text{Br}^-$

**FORMULA WEIGHTS:**

$\text{NaNO}_3$ : 85.0	$\text{NO}_3^-$ : 62.0049
$\text{Na}_2\text{SO}_4$ : 142.04	$\text{SO}_4^{2-}$ : 96.04
$\text{NaBr}$ : 102.89	$\text{Br}^-$ : 79.89
$\text{NaF}$ : 41.99	$\text{F}^-$ : 18.99
$\text{Na}_2\text{HPO}_4$ : 141.96	$\text{HPO}_4^{2-}$ : 95.96
$\text{NaNO}_2$ : 69.0	$\text{NO}_2^-$ : 46.0
$\text{NaCl}$ : 58.42	$\text{Cl}^-$ : 35.42

Mass of Compound needed to make 500mL of 1000mg/L sol'n:

(See Ashley's Lab Notebook #1 (Fall 2008-Summer 2009), page 24 for calculations)

$\text{NaNO}_3$  - 0.68543g

$\text{Na}_2\text{SO}_4$  - 0.739485g

$\text{NaBr}$  - 0.64395g

$\text{NaF}$  - 1.1056g

$\text{Na}_2\text{HPO}_4$  - 0.73969g

$\text{NaNO}_2$  - 0.75g

$\text{NaCl}$  - 0.824675g

*Procedures-*

**Individual Anion Standards:**

Transfer slightly over the desired weight in a 50mL beaker (on balance, tare beaker weight)

Place in the oven for 2 hours to dry

Desiccate until reaches room temperature

Using weigh paper, weight out mass of compound needed (see above) and transfer into 500mL volumetric flask. Use squirt bottle on weigh paper in order to ensure all mass is transferred.

Place stir bar in flask and stir at setting "3" until compound is completely dissolved.

Transfer, using funnel if needed, into 500mL plastic bottle.

Cap and invert bottle 3 times to ensure complete continuity of solution.

Place in fridge.

**Mixed Standard Solution A:**

In 100mL volumetric flask

Transfer 10mL of every anion desired

Dilute to 100mL with Milli-Q deionized water

Cover with Parafilm

Invert 3 times to ensure well-mixed

**Mixed Standard Solution B:**

In 100mL volumetric flask

Transfer 10mL of Mixed Standard Solution A

Dilute to 100mL with MilliQ deionized water

Cover with parafilm

Invert 3 times to ensure well-mixed

Using Mixed Standard Solutions A and B, calibration standards are prepared with milliQ deionized water in 100mL volumetric flasks (as illustrated in Table I below).

**Table I. Preparation of Anion Calibration Standards**

Standard	Anion concentration (mg/L)	mL of standard Solution/100mL
<b>Mixed Standard Solution A</b>		
1	25.0	25.0
2	10.0	10.0
3	3.0	3.0
<b>Mixed Standard Solution B</b>		
4	1.0	10.0
5	0.5	5.0
6	0.2	2.0
<b>1mg/L Standard (STANDARD 4)</b>		
7	0.1	10.0
8	0.05	5.0

*Preparing Water Samples for the IC*

**10X dilution:**

Using Eppendorf tubes.

Transfer 100  $\mu$ L of sample from 250mL plastic bottle and 900  $\mu$ L of milliQ deionized water.

Cap and shake to ensure well-mixed.

## Arsenic Sequential Extraction Procedure

### MATERIALS

50mL polypropylene centrifuge tubes, Teflon tip syringes for decanting, 200nm polycarbonate filters, Büchner Funnel, Aluminum Foil, Mortar and pestle, Shaker Plate, Centrifuge, Watch Glass, Hot Plate, Timer.

### CHEMICALS AND REAGENTS

1. Titanium (III) Chloride ( $\text{TiCl}_3$ , M.W. 154.26); 20% in 3% hydrochloric acid; Fisher AA3974330.
2. Water used to prepare the solution is milli-Q water (18.2 m $\Omega$ ).
3. Acid ( $\text{HCl}$  and  $\text{HNO}_3$ ) is at trace-metal grade.
4. All other chemicals are at ACS grade.
5. All solutions (except  $\text{NaHCO}_3$  solution) are degassed for 10 minutes with pure  $\text{N}_2$  and stored inside the anaerobic chamber.

#### Solution 1: 1M $\text{MgCl}_2$ , pH 8

$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  M.W.  $203.30\text{g}\cdot\text{mol}^{-1}$ ,  $1\text{M} \times 1\text{L} \times 203.30\text{g}\cdot\text{mol}^{-1} = 203.30\text{g}\cdot\text{L}^{-1}$

1. Add 203.30g  $\text{MgCl}_2$  into 1L volumetric flask, fill with about 600 ml Milli-Q water;
2. Place a pH meter in solution along with a stir bar, and adjust pH to 8;
3. Bring the total volume to 1 liter.

#### Solution 2: 1M $\text{NaH}_2\text{PO}_4$ , pH 5

$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  F.W.  $137.99\text{g}\cdot\text{mol}^{-1}$ ,  $1\text{M} \times 1\text{L} \times 137.99\text{g}\cdot\text{mol}^{-1} = 137.99\text{g}\cdot\text{L}^{-1}$

1. Add 137.99g  $\text{NaH}_2\text{PO}_4$  into 1L volumetric flask, fill with about 600 ml Milli-Q water.
2. Place a pH meter in solution along with a stir bar, and adjust pH to 5;
3. Bring the total volume to 1 liter.

#### Solution 3: 0.2M Ammonium Oxalate/oxalic acid, pH 3

$(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$  F.W.  $142.11\text{g}\cdot\text{mol}^{-1}$

$0.2\text{M} \times 1\text{L} \times 142.11\text{g}\cdot\text{mol}^{-1} = 28.42\text{g}\cdot\text{L}^{-1}$

Oxalic Acid M.W.  $134.0\text{g}\cdot\text{mol}^{-1}$

$0.2\text{M} \times 1\text{L} \times 134.0\text{g}\cdot\text{mol}^{-1} = 26.8\text{g}\cdot\text{L}^{-1}$

1. Add 28.42g Ammonium Oxalate into 1 L volumetric flask,
2. Fill the flask  $\frac{3}{4}$  of the volume with Milli-Q water. \*Always add acid to water\*
3. Add 26.8g oxalic acid
4. Place a pH meter in solution along with a stir bar, and adjust pH to 3;
5. Bring the total volume to 1 liter.

#### Solution 4: 1N $\text{HCl}$

trace metal grade  $\text{HCl}$ : 35% F.W. 36.46

Density of Conc.  $\text{HCl}$  =  $1.175\text{g}/\text{cm}^3$  at 35%,

$((35/100)(1000\text{mL})(1.175\text{g}/\text{mL}))/36.46\text{g}\cdot\text{mol}^{-1} = 11.28\text{M}$

$11.28x=1(1)$ ,  $X = 1/11.28$ ,  $X = 0.0886$  L, 88.6mL HCl per liter

1. Fill 1L volumetric flask  $\frac{3}{4}$  of the volume with Milli-Q water. \*Always add acid to water\*

3. Pour about 90 ml trace-metal grade HCl into an acid washed beaker. Transfer 88.6mL HCl into the flask. Do not pipette directly from the acid bottle!!!

3. Cap the flask and invert three time to mix.

4. Fill to 1 L with Milli-Q water

### **Solution 5: Ti(III)-citrate-EDTA & Bicarbonate solutions:**

Ti(III)-citrate-EDTA solution (1 liter) contains 0.05 M  $\text{TiCl}_3$ , 0.05 M  $\text{Na}_2\text{-EDTA}$ , and 0.05 M Na-citrate.

0.05M  $\text{TiCl}_3$

20% F.W. 154.26

Density =  $1.22\text{g/cm}^3$  at 20%,

$((20/100)(1000\text{mL})(1.22\text{g/mL}))/154.26\text{mol}^{-1} = 1.5817\text{M}$

$1.5817x=1(.05)$ ,  $X = .05/1.5817$ ,  $X = 0.0316$  L, 31.6 mL  $\text{TiCl}_3$  per liter

0.05M  $\text{Na}_2\text{-EDTA}$

$\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$  F.W.  $372.24\text{ g}\cdot\text{mol}^{-1}$ ,  $0.05\text{M} \times 1\text{L} = 0.05 \times 1 \times 372.24 = 18.612\text{ g}\cdot\text{L}^{-1}$

0.05M sodium citrate

F.W.  $294.10\text{ g}\cdot\text{mol}^{-1}$ ,  $0.05\text{M} \times 1\text{L} = 0.05 \times 1 \times 294.10 = 14.705\text{ g}\cdot\text{L}^{-1}$

Fill 1L volumetric flask  $\frac{3}{4}$  of the way full with Milli-Q water. \*Always add acid to water\*

Add 31.6mL  $\text{TiCl}_3$  + 20.81g  $\text{Na}_4\text{-EDTA}$  + 14.705g sodium citrate to 1L volumetric flask.

Place a pH meter in solution along with a stir bar.

The solution is degassed with  $\text{N}_2$  and pH is adjusted to 7.

Fill to 1L with Milli-Q water.

### **Solution 6 : $\text{NaHCO}_3$ solution**

Bicarbonate solution (100 ml) contains 1.0M  $\text{NaHCO}_3$ .

F.W.  $84.01\text{ g}\cdot\text{mol}^{-1}$ ,  $1\text{M} \times 100\text{mL} = 1 \times 0.1 \times 84.01 = 8.401\text{ g}\cdot\text{L}^{-1}$

1. 8.401g of  $\text{NaHCO}_3$  is weighed and moved to the anaerobic chamber.

2. Prepare the solution inside the anaerobic chamber using  $\text{N}_2$ -degassed milli-Q water.

3. Solution 5 and 6 are added together in 10:1 volume ratio (e.g., 40 ml Ti(III)-citrate-EDTA and 4 ml  $\text{NaHCO}_3$  solution).

### **FIELD SAMPLING**

1. Immediately after retrieving sediment packs from the well, place the pack into an anaerobic jar, and purge the jar with  $\text{N}_2$  for three minutes.

2. Store the jar at  $4^\circ\text{C}$  until returned to lab.

3. Back to the lab, move the jar into the anaerobic chamber. Please open the jar when transporting the jar through the airlock. The vacuum can break a closed jar.



4. Inside the anaerobic chamber, transfer 0.4 g into a sterile Eppendorf tube and the rest to other Eppendorf tubes.
5. Inside the anaerobic chamber, centrifuge at 11,000g for 25 minutes (~13,000 rpm, Spectrafuge 16M), remove water from sample using pipetting/decanting. Please balance the rotor before centrifuging.
6. Inside anaerobic chamber, transfer Eppendorf tubes into a labeled Ziploc bags. Store the bags in -80°C freezer until analysis.

## ANALYSIS

### 1. Magnesium Step – Targeting ionically bound arsenic

1. 0.4g of sediment is homogenized inside anaerobic chamber with mortar and pestle until near uniform consistency (<125 microns).
2. Add sediment into 50mL centrifuge tube (Polypropylene).
3. Add 40mL of 1M  $\text{MgCl}_2$  at pH 8. Note: sediment-to-extractant ratios of 1:1000 (0.4g to 40mL) are used for each step
4. Tumble-shake sample for 2 hours.
5. Centrifuge for 25 minutes at 11,000g, decant supernatant using a syringe; filter the supernatant inside the syringe using 0.2  $\mu\text{m}$  polycarbonate filter into a sampling vial.
6. Repeat step 3 to 5.
7. Repeat step 3 to 5 using Milli-Q water.
8. Acidify the filtrate in the sampling vials with trace metal grade HCl (final concentration 24 mM).

### 2. $\text{PO}_4$ Step – Targeting strongly adsorbed arsenic

1. Inside the anaerobic chamber, add 40mL of 1M  $\text{NaH}_2\text{PO}_4$  at pH 5, to the remaining residue.
2. Tumble-shake suspension for 16 hours.
3. Centrifuge for 25 minutes at 11,000g, decant supernatant using a syringe; filter the supernatant inside the syringe using 0.2  $\mu\text{m}$  polycarbonate filter into a sampling vial.
4. Repeat step 1 to 3, but shake the suspension for 24 hours.
5. Repeat step 1 to 3 using Milli-Q water and shake the suspension for 30 min.
6. Acidify the filtrate in the sampling vials with trace metal grade HCl (final concentration 24 mM).

### 3. HCl Step – targeting As co-precipitated with AVS (acid volatile sulfide), carbonates, Mn oxides, and very amorphous Fe oxyhydroxides

1. Inside the anaerobic chamber, add 40mL of 1N HCl to remaining residue
2. Tumble-shake suspension for 1 hour.
3. Centrifuge for 25 minutes at 11,000g, decant supernatant using a syringe; filter the supernatant inside the syringe using 0.2  $\mu\text{m}$  polycarbonate filter into a sampling vial.
4. Repeat step 1 to 3 using Milli-Q water.
5. Acidify the filtrate in the sampling vials with trace metal grade HCl (final concentration 24 mM).

#### **4. Ox Step – targeting As co-precipitated with amorphous Fe oxyhydroxides**

1. Inside the anaerobic chamber, add 40mL of 0.2M ammonium oxalate/oxalic acid at pH 3, to remaining residue.
2. Cover tube with aluminum foil, tumble shake suspension for two hours.
3. Centrifuge for 25 minutes at 11,000g, decant supernatant using a syringe; filter the supernatant inside the syringe using 0.2  $\mu\text{m}$  polycarbonate filter into a sampling vial.
4. Repeat step 1 to 3 using Milli-Q water.
5. Acidify the filtrate in the sampling vials with trace metal grade HCl (final concentration 24 mM).

#### **5. Ti(III)/Citrate/EDTA/bicarbonate extraction – targeting As co-precipitated with crystalline Fe oxyhydroxides**

1. Inside the anaerobic chamber, add 40 ml Ti(III)-citrate-EDTA solution and 4 ml  $\text{NaHCO}_3$  solution.
2. Tumble-shake suspension for 2 hours.
3. Centrifuge for 25 minutes at 11,000g, decant supernatant using a syringe; filter the supernatant inside the syringe using 0.2  $\mu\text{m}$  polycarbonate filter into a sampling vial.
4. Repeat step 1 to 3.
5. Repeat step 1 to 3 with Milli-Q water.
8. Acidify the filtrate in the sampling vials with trace metal grade HCl (final concentration 24 mM).

#### **6. $\text{HNO}_3$ Step – targeting As co-precipitated with pyrite and amorphous $\text{As}_2\text{S}_3$**

1. Inside the anaerobic chamber, add 40mL of 16N  $\text{HNO}_3$  to the remaining residue
2. Tumble-shake suspension for 2 hours.
3. Centrifuge for 25 minutes at 11,000g, decant supernatant using a syringe; filter the supernatant inside the syringe using 0.2  $\mu\text{m}$  polycarbonate filter into a sampling vial.
4. Repeat step 1 to 3 twice.
5. Repeat step 1 to 3 using Milli-Q water.

#### **7. Hot $\text{HNO}_3$ step – targeting orpiment and remaining recalcitrant As minerals** EPA method 3050B

1. For each digestion procedure, weigh to the nearest 0.01 g and transfer a 1-2 g sample (wet weight) or 1g sample (dry weight) to a digestion vessel. For samples with high liquid content, a larger sample size may be used as long as digestion is completed.
2. For the digestion of samples for analysis by GFAA or ICP-MS, add 10 mL of 1:1  $\text{HNO}_3$ , mix the slurry, and cover with a watch glass or vapor recovery device. Heat the sample to  $95^\circ\text{C} \pm 5^\circ\text{C}$  and reflux for 10 to 15 minutes without boiling.
3. Allow the sample to cool, add 5 mL of concentrated  $\text{HNO}_3$ , replace the cover, and reflux for 30 minutes. If brown fumes are generated, indicating oxidation of the sample by  $\text{HNO}_3$ , repeat this step (addition of 5 mL of conc.  $\text{HNO}_3$ ) over and over until no brown fumes are given off by the sample indicating the complete reaction with  $\text{HNO}_3$ .

4. Using a ribbed watch glass or vapor recovery system, either allow the solution to evaporate to approximately 5 mL without boiling or heat at  $95^{\circ}\text{C} \pm 5^{\circ}\text{C}$  without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.
5. After the sample has cooled, add 2 mL of water and 3 mL of 30%  $\text{H}_2\text{O}_2$ .
6. Cover the vessel with a watch glass or vapor recovery device and return the covered vessel to the heat source for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides and the vessel cools. Continue to add 30%  $\text{H}_2\text{O}_2$  in 1-mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged.
7. Cover the sample with a ribbed watch glass or vapor recovery device and continue heating the acid-peroxide digestate until the volume has been reduced to approximately 5mL or heat at  $95^{\circ}\text{C} \pm 5^{\circ}\text{C}$  without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.
8. After cooling, dilute to 100mL with water. Particulates in the digestate should then be removed by filtration, by centrifugation, or by allowing the sample to settle. The sample is now ready for analysis by GFAA or ICP-MS.

### Iron Sequential Extraction Procedure

#### Solutions:

0.2M Sodium citrate plus 0.35M Acetic acid - pH 4.8

Sodium dithionite (also called, Sodium hydrosulfite)

Ferrozine

10% HA (Hydroxylamine hydrochloride)

0.5M HCl

#### Materials:

Rotary shaker

Serum Vials

Rubber Stoppers

Spectrophotometer

#### Procedure:

1. Total reactive Fe (amorphous+crystalline Fe(III) oxides and surface-associated Fe(II))
  - Add 0.1-1g of wet sediment to 10mL of 0.2M Sodium citrate plus 0.35M Acetic acid solution at pH 4.8 (pH adjusted with 6N HCl).
  - Immediately add 0.5g of Sodium dithionite and cap vial.
  - Place on rotary shaker for 1 hour.
  - Remove from shaker to hood, uncap and allow to sit overnight.
  - Next day, add 5-10mL of Ferrozine plus 0.25mL of 10% HA
  - Let sit overnight
  - Next day, read  $A_{562}$  of the ferrozine-extract mixture using spectrophotometer.
2. Poorly-crystalline Fe(III) and surface-associated Fe(II)
  - In the anaerobic chamber, add 0.1-1g of wet sediment to 10mL of 0.5M HCl, cap, place on shaker for 1 hour.
  - Remove from shaker and let sit for 2 hours.
  - Add 0.5mL of extract to 5mL Ferrozine and immediately withdraw 1 mL using pipette to measure  $A_{562}$ . (Giving you Fe(II) content of the extract)
  - Add 0.25mL of 10% HA to remaining mixture, wait 15 minutes
  - Read  $A_{562}$  again. (Giving you Total Fe(II) plus Fe(III) content of the extract).
  - Fe(III) can be calculated from the difference between total Fe and Fe(II).

## Sulfide Sequential Extraction Procedure

### Solutions:

7.5mM Zinc acetate, pH 9  
6N HCl  
Tin Chloride  
Conc. HCl  
Ethanol  
1M Cr(II) -- Created from Jones Reducer  
*Jones Reducer:*  
Cr(III) salt  
2% Mercuric chloride  
Conc. Nitric Acid  
20-30 mesh Zinc

### Materials:

Reducer Column  
Glass bottles  
Needles - 6" and short  
One-way or three-way valves  
Tubing  
N<sub>2</sub>  
Septums  
Septum bottles

### Pre-Experiment Procedures:

#### *Cr(III) Solution*

- Dissolve 133g of reagent-grade CrCl<sub>3</sub>·6H<sub>2</sub>O in 500mL of 0.1M HCl.
- Pass solution through Jones Reducer, described below.

#### *Jones Reducer*

- Add 200mL of 2% Mercuric chloride to 1mL of conc. Nitric Acid, finally to 200g of 20-30 mesh Zinc in a beaker.
- Stir mixture for 10 minutes.
- Decant solution and wash zinc with DI water 3 times.
- Fill reducer column with zinc (zinc should be bright silver) until packed.
- Wash column with 500mL of DI water.
- Pour Cr(III) solution through reducer column, into glass bottle, being purged constantly with N<sub>2</sub>.

### Procedure:

- Combine 1.5 SnCl<sub>2</sub> and sample into purging septum bottle. Cap. Set up needles, valve, and tubing connections with AVS sink bottle (containing 10mL of zinc acetate) and N<sub>2</sub> source.

- Once flow rate of N<sub>2</sub> is constant at a rate of ~2mL/min, add 30mL of N<sub>2</sub> purged HCl into sample septum bottle.
- Let react for 2 hours.
- After 2 hours, seal valves on each bottle and remove AVS sink bottle and replace with CVS sink bottle (also containing 10mL of zinc acetate). Open valves and add 10mL of ethanol to sample bottle, wait 5 minutes. Next, inject 30mL of 1M Cr(II) Solution and 15M conc. HCl.
- Let react for 1 hour.
- Using the sulfide spectrophotometer procedure, read concentration for AVS and CRS sinks.

### Ferrous Iron Spectrophotometer Procedure

**Ferrozine Reagent:** (FW 492.47, 97%, Aldrich #16,060-1):  $10^{-2} \text{ mol}\cdot\text{l}^{-1}$  prepared in an ammonium acetate ( $\text{CH}_3\text{COONH}_4$ , Aldrich #37,233-1, 99.999%) solution of  $10^{-1} \text{ mol}\cdot\text{l}^{-1}$ .

#### Ferrous Iron Standards

1. Stock Solution A ( $100 \text{ mM Fe}^{2+}$ ). Weight  $0.69505 \text{ g FeSO}_4\cdot 7\text{H}_2\text{O}$  and add into a  $25 \text{ ml}$  volumetric flask. Bring the final volume to  $25 \text{ ml}$  using Milli-Q water ( $18.2 \text{ m}\Omega$ ). ( $25\text{ml}\times 100\text{mM}\times 278.02\text{g}\cdot\text{mol}^{-1} = 0.69505 \text{ g}$ ).
2. Prepare the standards according to the following table:

Standard	mM	Mixing	
		ml of Standard	mL of water
B	10	10 / A	90
C	1	1 / A	99
D	0.5	5 / B	95
E	0.1	1 / B	99
F	0.05	5 / C	95
G	0.01	1 / C	99
H	0.005	1 / D	99
I	0.001	1 / E	99
J	0.0005	1 / F	99

#### Procedure

1. Power Beckman Coulter DU 530 Spectrophotometer by pressing ON/OFF button on the back left side; let warm up at least five minutes before analysis.
2. Add  $1 \text{ ml}$  of filtered samples or standards to  $100 \mu\text{l}$  of reagent A.
3. Incubate the mixture at room temperature for 3 minutes.
  - User Program
  - Program 2 – Fix wavelength: Ferrous iron
  - 2
  - Enter
  - Wavelength 562 will pop up automatically
  - Blank – DI water

\*don't need to reblank between samples\*

#### References:

Viollier, et al. 2000. The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. *Applied Geochemistry* 15:785-790.

## Sulfide Spectrophotometer Procedure

**Reagents:** Hach Sulfide 1 Reagent and Sulfide 2 Reagent

### Sulfide Standards

1. Weight about one gram  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  (M.W.  $240.18 \text{ g} \cdot \text{mol}^{-1}$ ), dry using a piece of ChemWipe paper.
2. Stock Solution A ( $100 \text{ mM S}^{2-}$ ). Weight  $0.60045 \text{ g Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  and add into a  $25 \text{ ml}$  volumetric flask. Bring the final volume to  $25 \text{ ml}$  using Milli-Q water ( $18.2 \text{ m}\Omega$ ). ( $25\text{ml} \times 100\text{mM} \times 240.18\text{g} \cdot \text{mol}^{-1} = 0.60045 \text{ g}$ ).
3. Prepare the standards according to the following table:

Standard	mM	Mixing	
		ml of Standard	mL of water
B	10	10 / A	90
C	1	1 / A	99
D	0.5	5 / B	95
E	0.1	1 / B	99
F	0.05	5 / C	95
G	0.01	1 / C	99
H	0.005	1 / D	99
I	0.001	1 / E	99
J	0.0005	1 / F	99

### Procedure

1. Turn on Beckman Coulter DU 530 Spectrophotometer by pressing ON/OFF button on the back left side; let warm up for at least five minutes before analysis.
2. Inside  $1.5 \text{ ml}$  Eppendorf tubes, add  $1 \text{ ml}$  of filtered samples or standards,  $50 \mu\text{l}$  of reagent 1, and  $50 \mu\text{l}$  of reagent 2. Mix the solution by inverting the tubes on a rack three times.
3. Incubate the mixture at room temperature for 3 minutes.
4. Spectrophotometer Steps:
  - User Program
  - Program 3 – Fix wavelength: Sulfide
  - 3
  - Enter
  - Wavelength 665 will pop up automatically
  - Blank – DI water

\*don't need to reblank between samples\*

- run standard samples from lowest concentration to highest concentration.

\*when inserting sample container, be sure **clear** plastic portion points to the sides, keep opaque side facing front a back. Handle opaque sides, not clear sides\*



APPENDIX B  
GROUNDWATER SAMPLING PROCEDURE

## Groundwater Sampling Procedure

### MATERIALS

Water Level

Pump

Power (extension cord, battery charger, battery, pump voltage meter)

Degassing Setup (large PVC tube, two grey PVC ends with valves, small N<sub>2</sub> tank)

Gas Sampling Station (gas sampling toolbox, 2 60 mL syringes with tubing for samples from bailer, 2 N<sub>2</sub> purged glass bottles)

Water Sampling Station (large black toolbox: spectrophotometer, pipets, pipet tips, falcon tubes, gloves. Yellow toolbox: trace metal grade nitric and hydrochloric acid, 250 EDTA, Sulfide reagent solutions, ferrous iron reagent powder packs)

Flow Cell (Glass Mason Jar, Temp gauge, pH/Conductivity meter)

Water Sampling (Sample bottles, falcon tubes, water filter, tubing attachment from pump to filter, timer, 2L coke bottle)

Carboy of DI water

### PROCEDURE

*Take Water Level Measurement*

*Set up Power*

*Set up degassing tube*

*Set up Gas Sampling Station*

*Set up Water Sampling Station*

*Set up flow cell*

*Take Gas Sample:*

Pull up bailer from within PVC

Immediately insert tubes attached to 60mL syringes into first opening in bailer

Collect water from section into syringes

Remove air from syringes and close syringe valve

Take off attached tubes

Pull out bailer from PVC and purge with N<sub>2</sub> in degassing tube for 2 minutes

Attach needle to end of one syringe, open syringe valve, squirt out some water to remove air from needle tip

Record volume of water within syringe

Transfer water sample into 160mL glass bottle

Repeat procedure for second syringe

Shake bottle for 2-3 minutes to allow for gas and water to equilibrate

Attach needle to 10mL syringe

Flush needle with 1mL of gas from 160mL glass bottle

Take 10mL of gas from 160mL bottle and transfer into small glass bottle.

*Take Water Sample:*

Turn on pump

Set and record voltage  
 Remove air bubbles out of tubing  
 Place in 2L Coke bottle, begin timer  
 Record Flow Rate  
 Transfer tubing to Flow Cell  
 Turn on Temp Probe and pH/Conductivity meter  
 Record Temp, pH, and Conductivity  
 Put on gloves  
 Hold Water filter upright (having the water flow from the bottom upwards)  
 Screw on tubing to bottom of water filter  
 Begin taking water samples  
 Rinse each bottle or falcon tube once  
 Fill bottles to top and cap  
 \*Sample water from as close to tip of water filter as possible, avoid as much contact with air as possible\*  
 Turn off pump  
 Pull up pump from well  
 Pump through DI water from carboy to rinse pump

*Spectrophotometer:*

*Need:* DR2000 spectrophotometer, timer, reagents (sulfide, ferrous Fe), DI water, waste bucket, falcon tubes with water samples

Decant 25mL of sample from one falcon tube (label H<sub>2</sub>S).  
 Add 1mL Sulfide 1 Reagent and then 1mL Sulfide 2 Reagent. Cap. Mix Well.  
 Decant 25mL of sample from another falcon tube (label Fe<sup>2+</sup>).  
 Add 1 Ferrous Fe Reagent Pillow Packet into tube. Cap. Mix Well.  
 Turn on spectrophotometer. Enter Method 690 (program for sulfide), press Read/Enter; follow instructions and rotate dial to 665nm; Press Read/Enter.  
 To blank for sulfide, fill sample cell with 25mL of DI water. Place into sample holder, close lid, press Zero.  
 Pour into waste bucket, add 25mL of sample from falcon tube H<sub>2</sub>S into sample cell, press Read/Enter.  
 Record concentration output on screen.  
 Empty sample into waste bucket and wash sample cell out with DI water.  
 Begin again entering Method 255 for Fe<sup>2+</sup>. Press Read/Enter and rotate the dial to 510nm; press Read/Enter.  
 The blank for Fe<sup>2+</sup> is untreated well water collected in another falcon tube.  
 Pour 25mL of this water into the sample cell, place in sample holder, close lid, hit Zero.  
 Pour into waste bucket, add treated sample, in falcon tube Fe<sup>2+</sup>, into sample cell. Press Read/Enter.  
 Record concentration output on screen. Empty sample into waste bucket and wash sample cell out with DI water.

*Treating Water Samples:*

## Chemical Treatment:

- Add 0.5 mL trace metal grade  $\text{HNO}_3$  to the 2 60mL polyethylene bottle.
  - Arsenic: add 100ul EDTA to 2 of the 3 opaque polyethylene bottles. Add 130uL trace metal grade  $\text{HCl}$  to marked 30mL bottle.
- Chill all bottles on ice.

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